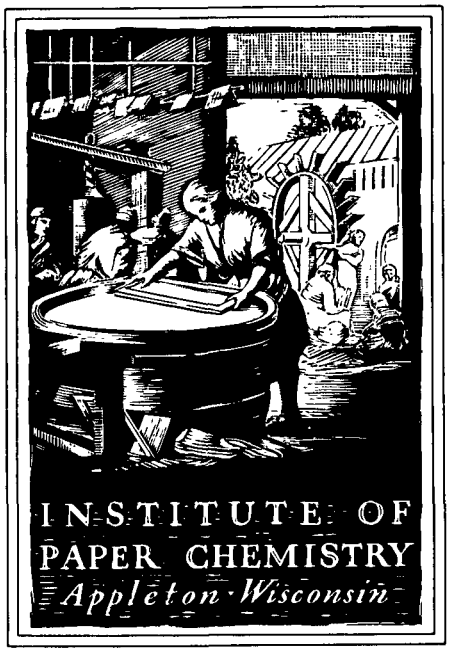
3223

PROJECT ADVISORY COMMITTEE

Subcommittee on

Forest Genetics



IPC STAFF STATUS REPORTS

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Your advice and suggestions on any of the projects will be most welcome.

FOR MEMBER COMPANIES ONLY

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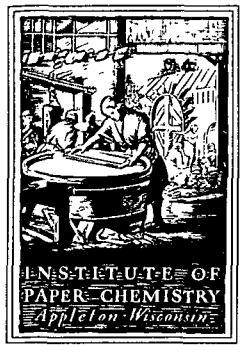

THE INSTITUTE OF PAPER CHEMISTRY
Post Office Box 1039
Appleton, Wisconsin 54912
Phone: 414/734-9251
Telex: 469289

September 30, 1987

TO: Members of the Forest Genetics Project Advisory Committee

As indicated in my recent letter, I am forwarding a detailed agenda for the fall meeting of the Forest Genetics Project Advisory Committee. Enclosed also is some advance reading material, our most recent status report. Please use the enclosures to prepare for participation.

All here are battling on a number of fronts - long-range planning, curriculum revision, student recruiting, service and consulting arrangements, teaching, and what not. Amidst the fracas, however, we are summarizing data and preparing for the fall PAC meeting.

We look forward to sharing new developments and plans on October 26 and 27. Please remember to register, if you have not already done so. Be certain also to circulate the enclosures to other colleagues that plan to participate.

Many thanks and best regards.

Sincerely,

Ronald J. Dinus
Director
Forest Biology Division

RJD/sjb
Enclosure
Copies to: Dr. Stanley Krugman, USDA Forest Service
Dr. Ralph Mott, North Carolina State University

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AGENDA

FOREST GENETICS PROJECT ADVISORY COMMITTEE

October 26-27, 1987
The Institute of Paper Chemistry
Continuing Education Center
Appleton, Wisconsin

Monday, October 26

8:00 a.m.	Laboratories open and personnel available	
NOON	Lunch, CEC Dining Room	
1:00 p.m.	Opening Remarks Welcome/Introductions Overview Review of PAC Recommendations	Dinus
1:15	Initiation and Maintenance of Embryogenic Callus Immature Explants, Pines Frequency of Initiation Characteristics of Callus Prognosis	Becwar
1:40	Douglas-Fir and Mature Explants Frequency of Initiation Characteristics of Callus Prognosis and New Experiments	Nagmani
2:00	Embryo Development and Maturation Somatic Embryos White Spruce, Immature and Mature (15) Loblolly Pine, Immature (15)	 J. Wyckoff Kroll
2:30	Zygotic Embryos, Loblolly Pine Results Synthesis	Wann
2:50	Biochemistry of Development Biochemical Events (20) Protein Composition and Comparisons (15)	 Johnson Feirer
3:25	Coffee Break	
3:45	Fidelity and Performance Growth and Development; Isozyme Analyses	Johnson
4:05	Exploratory Research Suspension Cultures (15) Protoplasts (15)	 Verhagen Nagmani
4:35	Summary and Discussion (25)	Dinus
5:00	Cocktails and Dinner, CEC Dining Room	
7:00	Open Discussion	

AGENDA (Contd.)

Tuesday, October 27

7:30 a.m.	Breakfast, CEC Dining Room	
8:00	Agenda for Morning	Chairman/Dinus
8:15	Discussion	Committee
9:45	Coffee Break	
10:00	Discussion/Deliberations	Committee
11:15	Closing Remarks	Chairman/Dinus
11:30	Adjournment/Lunch, CEC Dining Room	

NEXT MEETING: March 30-31, 1988

FOREST GENETICS

Project Advisory Committee

Mr. Edwin G. Owens (Chairman) - 6/89*
Forest Research Director
Westvaco Corporation
Timberlands Division
Forest Research
P. O. Box 1950
Summerville, SC 29484-1950
(803)871-5000

Dr. Jan Bonga - 6/88
Canadian Forestry Service
Maritime Forest Service Center
P. O. Box 4000
College Hill
Fredericton, NB E3B 5P7
CANADA
(506)452-3500

Dr. Michael S. Greenwood - 6/89
Professor of Forest Resources
University of Maine at Orono
Department of Forest Biology
201 Nutting Hall
Orono, ME 04469
(207)581-2881

Dr. William A. Kindler, Jr. - 6/89
Vice President of Sales, Printing Papers
Crown Zellerbach Corporation
Communications Papers West
349 N.W. 7th Avenue
Camas, WA 98607
(206)834-4444

Mr. Robert Lazar - 6/89
Manager, Project Planning
Union Camp Corporation
R&D Division
P. O. Box 3301
Princeton, NJ 08543-3301
(609)896-1200

Mr. Gregory N. Leach - 6/89
Genetics Research Forester
Champion International Corporation
Southern Timberlands Division
P. O. Box 87
Cantonment, FL 32533
(904)968-2121

*date of retirement
9/28/87 sjb

Dr. Rex McCullough - 6/88
Team Leader
Weyerhaeuser Company
Forestry Resources Division
Tacoma, WA 98477
(206)924-6115

Mr. Billy Wayne McIntyre - 6/89
Manager of Operational Planning
and Appraisal
MacMillan Bloedel Inc.
P. O. Box 336
Pine Hill, AL 36769
(205)963-4391

Mr. Sharon R. Miller - 6/90
Director, Woodlands & Wood Products
Chesapeake Corporation
19th & Main Streets
P. O. Box 311
West Point, VA 23181
(804)843-5278

Mr. Martin P. Rech - 6/89
Services Manager
Consolidated Papers, Inc.
P. O. Box 158
Rhinelander, WI 54501
(715)369-4770

Mr. James Schuler - 6/90
Technical Services Forester
Green Bay Packaging Inc.
Southern Woodlands Division
P. O. Box 711
Morrilton, AR 72110
(501)354-2461

Dr. Ronald A. Woessner - 6/90
Manager of Forest Productivity
The Mead Corporation
Georgia Kraft Division
P. O. Box 8847
Columbus, GA 31908
(404)323-1863

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

Status Report

to the

FOREST GENETICS

PROJECT ADVISORY COMMITTEE

Project 3223

THE MASS PRODUCTION OF CONIFERS

October 26-27, 1987

PROJECT SUMMARY FORM

DATE: September 28, 1987

PROJECT NO. 3223 - Mass Production of Conifers

PROJECT STAFF: M. Becwar*, R. Dinus, R. Feirer, M. Johnson, R. Nagmani, S. Verhagen, S. Wann*

PROJECT OBJECTIVE/GOAL:

Overall - Mass production of genetically improved conifers.

Near-term - Develop procedures for producing plantlets from single cells or small groups of cells.

PROJECT RATIONALE:

Major increases can be obtained in tree growth, quality, and uniformity via rapid, low-cost methods for mass cloning of "elite" trees. Reliable cell and tissue culture systems will also open the way for genetic engineering, and production of new genetic combinations having exceptional growth, increased pest resistance, special fiber qualities, and enhanced site and/or climatic adaptability. Screening for and selecting useful variants in culture could also lower costs and raise efficiency of conventional tree breeding. Cell and tissue culture thus form the basis for second-generation technologies, which could improve upon existing technologies or greatly accelerate their pace.

CURRENT FISCAL YEAR BUDGET: $600,000

SUMMARY OF RESULTS SINCE LAST REPORT: (March 1987 - September 1987)

Methods previously developed for initiating callus, growing cells in suspension, and maintaining callus and cell lines remain in use or are being refined. Results from past work on nonconifer model systems, natural embryo

*Industrial Research Fellow

SUMMARY OF RESULTS SINCE LAST REPORT (Contd.)

development, and organogenesis have been integrated and are being applied to the Norway spruce model system and target species. The most reliable biochemical and histological markers are being used to screen cultures for embryogenic potential, and monitor effects of modified or new protocols.

Embryogenesis in the model system is now controlled and reproducible. Initiation of embryogenic callus from both immature and mature embryos is straightforward; embryo numbers can be quantified and seedlings recovered. Increased embryo numbers and development have been noted in suspension cultures, and good yields of viable protoplasts have been obtained. Experiments with the model system now use mature embryos, thus permitting year-round work. Similar progress has been made with white spruce, a commercially important species.

Though seedlings have been recovered, development/maturation of spruce somatic embryos remains difficult, and increasing efficiency of these steps remains a major effort. Results from earlier work on biochemistry of nonconifer model systems are proving helpful. Also, use of abcisic acid in development media has given positive results. Such refinements show potential for increasing seedling recovery, and results are being extended to target species as quickly as experiments are completed. Spruce seedlings recovered earlier entered dormancy and renewed vegetative growth in synchrony with their zygotic counterparts.

Histological assays have shown that spruce embryogenic callus originates from the few outermost cell layers of the hypocotyl. Accordingly, work with more mature spruce materials is using tissues similar to those in hypocotyls.

Somatic embryogenesis was obtained in white pine some months ago. The callus is similar to that of spruces, but origin and factors promoting formation are different. In white pine, embryogenic callus forms from the suspensor region, rather than from the hypocotyl as in spruce. In addition, initiation frequencies appear to vary with donor tree, suggesting genetic differences in embryogenic potential. Progress on development/maturation has been slow but positive. Lessons from the white pine experience are facilitating work on target species.

Embryogenic callus has also formed in cultures of pond and loblolly pines. Initiation frequencies have been low and variable. Even so, callus has been obtained from a variety of sources, including winter collections from South America and summer collections from the southeastern U.S. All lines are phenotypically and biochemically similar to spruce embryogenic callus. More recently, initiation was extended to pitch x loblolly pine hybrids. The method is thus reproducible, and appears generally applicable to pine species. Current efforts are focused on increase and development/maturation.

Status of Douglas-fir remains much the same. Embryogenic callus can be initiated, but not at will. Initiation frequencies are low. Increase is difficult and development/maturation has not been achieved. Experiments will continue over the winter months with stored cones and collections from New Zealand.

Biochemical/molecular tests of differences between embryogenic and nonembryogenic callus continued. A low molecular weight protein was found in most nonembryogenic calli, but was absent from comparable embryogenic cultures. The protein apparently is associated with differences in chloroplast development described earlier. In addition, techniques for isolating, purifying, and

characterizing both RNA and DNA have been developed or refined for use in assessing similarities/differences between embryogenic and nonembryogenic calli, immature and mature explants, and somatic and zygotic seedlings.

Work, on all fronts, over the last six months has proceeded and will continue for the immediate future in accordance with the plan approved last spring.

SHORT TERM GOALS:

Goals for Remainder of FY 87-88

1. Refine protocols for initiating embryogenic callus in target species. Exploit results from latest work on explant development stages and capitalize on findings from other laboratories. Characterize and compare cultures, using best biochemical markers and analyses.

2. Accumulate baseline data on histological, biochemical, and molecular characteristics of somatic and zygotic embryogenesis. Use biochemical markers, related biochemical analyses, and molecular tools to identify embryogenic potential, facilitate initiation of embryogenic callus, and improve development/maturation.

3. Improve protocols for increasing development/maturation frequencies, raising efficiency of conversion to seedlings, and providing sufficient material for replicated greenhouse trials. Develop and test biochemical and molecular tools for evaluation of fidelity and performance.

4. Increase ability to obtain and mature somatic embryos in alternative culture systems. Exploit recent progress with suspension cultures. Concentrate on systems well-suited to mass propagation.

SHORT TERM GOALS (Contd.)

5. Continue work on protocols for obtaining embryogenic callus from more mature explants. Determine culture factors and explant characteristics that must be altered, and evaluate procedures for changing them.

6. Execute exploratory research on origin of embryogenic callus, regeneration from single cells or protoplasts, promising molecular techniques, and genetic transformation.

7. Secure additional loblolly pine and Douglas-fir explant sources from southern hemisphere. Maintain potential for year-round experimentation.

8. Provide for prompt publication of findings.

COOPERATIVE INVESTIGATIONS:

1. North Carolina State University - Cooperative evaluation with Dr. R. Mott and Dr. H. Amerson of procedures for initiating embryogenic callus cultures of loblolly pine, Norway spruce, and white spruce.

2. Ohio State University, Wooster - Dr. Kriebel supplied immature white pine cones for initiation trials, and assisted in preparation of two manuscripts.

3. Williams College/Merrell-Dow Pharmaceutical Co. - Cooperative study with Dr. Robert Slocum (Williams) plus Drs. A. Bitonti and P. McCann (Merrell-Dow) of polyamine metabolism, and joint preparation of resultant manuscript.

4. International Forest Seed Company - Supply of "rejuvenated" loblolly pine material by Dr. S. Foster for experiments on initiation of embryogenic callus from mature explants.

5. University of Cincinnati - Joint assay with Dr. J. Caruso of endogenous hormone levels, principally ABA and IAA, in embryogenic and nonembryogenic calli. Arrangements now being finalized.

RELATED STUDENT RESEARCH:

Completed in 1987

Tyrone Cornbower - M.S., Independent Study, entitled "Response of white spruce to mechanical pulping following hemicellulose hydrolysis."

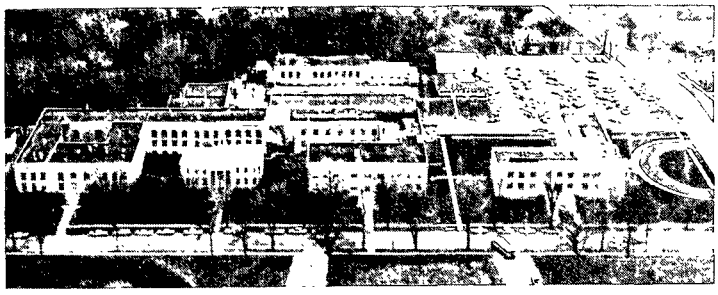
Luke Nealey - Ph.D. Program, Organic Chemistry Orientation, entitled "Isolation and characterization of xyloglucan from suspension cultured loblolly pine cell medium."

In Progress

Michael Bogenschutz - M.S., Independent Study, entitled "Electroporation-mediated genetic transformation of Norway spruce cells."

Russ Feirer - Ph.D. Program, Biochemical Orientation, entitled "Biochemical and molecular studies of plant development." In cooperation with University of Wisconsin, Madison.

Note: Several students in the class entering this fall, have expressed an interest in Project 3223.



THE INSTITUTE OF PAPER CHEMISTRY, APPLETON, WISCONSIN

FOREST GENETICS PROJECT ADVISORY COMMITTEE

HANDOUTS

October 26-27, 1987

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NEXT MEETING: March 30-31, 1988

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Project Advisory Committee

Mr. Edwin G. Owens (Chairman) - 6/89*
Forest Research Director
Westvaco Corporation
Timberlands Division
Forest Research
P. O. Box 1950
Summerville, SC 29484-1950
(803)871-5000

Dr. Jan Bonga - 6/88
Canadian Forestry Service
Maritime Forest Service Center
P. O. Box 4000
College Hill
Fredericton, NB E3B 5P7
CANADA
(506)452-3526

Dr. Michael S. Greenwood - 6/89
Professor of Forest Resources
University of Maine at Orono
Department of Forest Biology
201 Nutting Hall
Orono, ME 04469
(207)581-2881

Dr. Walter Jarck - 6/90
Corporate Director
Forest Resources
Georgia-Pacific Corporation
133 Peachtree Street, N.E.
P. O. Box 105605
Atlanta, GA 30348-5605
(404)521-5103

Mr. Robert Lazar - 6/89
Manager, Project Planning
Union Camp Corporation
R&D Division
P. O. Box 3301
Princeton, NJ 08543-3301
(609)896-1200

Mr. Gregory N. Leach - 6/89
Research & Development Manager
Champion International Corporation
Southern Timberlands Division
P. O. Box 87
Cantonment, FL 32533
(904)968-2121

*date of retirement
10/12/87 sjb

Dr. Rex McCullough - 6/88
Team Leader
Weyerhaeuser Company
Forestry Resources Division
Tacoma, WA 98477
(206)924-6115

Mr. Billy Wayne McIntyre - 6/89
Manager, Land Management
MacMillan Bloedel Inc.
P. O. Box 336
Pine Hill, AL 36769
(205)963-4391

Mr. Sharon R. Miller - 6/90
Director, Woodlands & Wood Products
Chesapeake Corporation
19th & Main Streets
P. O. Box 311
West Point, VA 23181
(804)843-5278

Mr. Martin P. Rech - 6/89
Timberlands Services Manager
Consolidated Papers, Inc.
P. O. Box 158
Rhinelander, WI 54501
(715)369-4770

Mr. James Schuler - 6/90
Technical Services Forester
Green Bay Packaging Inc.
Southern Woodlands Division
P. O. Box 711
Morrilton, AR 72110
(501)354-2461

Mr. Brian Stanton - 6/89
Geneticist
James River Corporation
349 N.W. 7th Avenue
Camas, WA 98607
(206)834-4444

Dr. Ronald A. Woessner - 6/90
Manager of Forest Productivity
The Mead Corporation
Georgia Kraft Division
P. O. Box 8847
Columbus, GA 31908
(404)323-1863

ATTENDANCE LIST

FOREST GENETICS PROJECT ADVISORY COMMITTEE

The Institute of Paper Chemistry
Appleton, Wisconsin

October 26-27, 1987

Committee

Mr. David Canavera
(substituting for Ed Owens)
Westvaco Corporation

Dr. Jan Bonga
Maritime Forest Service Center

Dr. Michael S. Greenwood
University of Maine at Orono

Mr. Brian Stanton
(permanently replacing Bill Kindler)
James River Corporation

Mr. Robert Lazar
Union Camp Corporation

Mr. Gregory N. Leach
Champion International Corporation

Dr. Jerry Pullman
(substituting for Rex McCullough)
Weyerhaeuser Company

Mr. B. W. McIntyre
MacMillan Bloedel Inc.

Mr. Sharon R. Miller
Chesapeake Corporation

Mr. Martin P. Rech
Consolidated Papers, Inc.

Mr. James Schuler
Green Bay Packaging Inc.

Dr. Ronald A. Woessner
(Acting Chairman)
The Mead Corporation
Georgia Kraft Division

Guests

Dr. H. (Doug) S. Dugal
James River Corporation

Dr. Stanley Krugman
U. S. Forest Service

Dr. Ralph Mott
North Carolina State University

Ms. Cheryl Talbert
Weyerhaeuser Company

IPC Personnel

Mike Becwar
John Carlson
Jud Conkey
Ron Dinus
Russ Feirer
Debra Hanson
Morris Johnson
Lynn Kroll
Nagmani Rangaswamy
Shirley Verhagen
Steve Wann
Gary Wyckoff
Judy Wyckoff
Richard Matula
Wendall Smith
Ronald Yeske

CODES

Tissue response and the results of many studies may be altered or complicated by the genetic differences between cell lines and/or the length of time in culture. To aid the reader (reviewer) in understanding, and the investigator in reporting/analyzing, it is important to be aware of the tissue source used for each study. An example and explanation of our standard tissue identification coding system is presented below; however, at times only part of the code may appear in a text.

All cell lines in excess of one year old:

Example: 20(NS 384-1)2E

20 = subcultured 20 times

NS = Norway spruce

384 = research plan (RP384)[a]

-1 = time of initiation or treatment identification

2 = line or genetic source, e.g., seedling No. 2

E = Immature embryo; explant type (only used if cell line derived from more than one explant within a research plan).

---

[a]Each experiment initiated by any team member has an approved research plan with an identifying number. The tissue source origin (clone, seed lot, etc.) and initiation date is recorded under that number in the investigator's IPC research notebook and is available in the Tissue Culture Research Plan files.

Cell lines less than one year old from immature cone collections:

Example: 5(LP6B)E - the RP No. is deleted and the letter within parentheses indicates cone source code.

Species Codes	Explant Codes
LP - loblolly pine	C = cotyledon
DF - Douglas-fir	H - hypocotyl
PP - pitch pine	B - bud
PO - pond pine	E - immature embryo
NS - Norway spruce	M - mature embryo
WP - white pine	N - nucellus
WS - white spruce	G - gametophyte

CONE SOURCES, 1986-1987

Species	Tissue Culture Code	Source	Industrial Codes
Douglas-fir	DF A	Weyerhaeuser	WTC-167
	DF B	Federal Way, WA	WTC-168
	DF C		WTC-169
	DF D		WTC-170
	DF E		WTC-171
	DF F		WTC-195
	DF G		WTC-196
	DF H		WTC-205
	DF I		WTC-207
Loblolly pine	LP A	Union Camp	10-1003 D-22 HQI
	LP B	Rincon, GA	10-1007 F-21 HQI
	LP C		10-1011 C-20 HQI
	LP D		10-1018 B-16 HQI
	LP E		10-1019 C-14 HQI
	LP F	Westvaco	7-34
	LP G	Summerville, SC	7-56
	LP H		11-9
	LP I		11-10
	LP J		11-16
Norway spruce	NS	Greenville, WI	--
	NSA	U. Arkansas Fayetteville, AR	
Pitch pine	PP A	Westvaco	1-417
	PP B	Summerville, SC	65
Pond pine	PO A	Union Camp	2-1011 R7
	PO B	Rincon, GA	10-760 Q6
	PO C		10-762 S9
	PO D		22-403 S10
White pine	WP A	Ohio State	1588
	WP B	Wooster, OH	1590
	WP I	Freedom, WI	--
	WP II		--
	WP III		--
White spruce	WS A	Greenville, WI	--
	WS B		--

ADDITIONAL CONE SOURCES, 1987

Species	Tissue Culture Code	Source	Industrial Codes
Douglas-fir	DF J DF K DF L DF M DF N	Weyerhaeuser Federal Way, WA	WTC-357 WTC-358 WTC-359 WTC-360 WTC-361
Loblolly pine	LP R	Westvaco, Summerville, SC	11-25
Pitch x loblolly pine hybrid	PL		65(♀) x LP(♂)
White spruce	WS 65	Oconto River Seed Orchard (U.S. Forest Service)	

STATISTICS

Where statistics beyond means and standard deviations (S.D.) were used in the evaluation of results to be presented, the data were subjected to analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test for multiple comparison of means. Values with a common superscript letter are not significantly different from each other ($P \leq 0.05$). The number of replications is indicated by N.

INTRODUCTORY REMARKS - RON DINUS

WELCOME

NEW FACES - MEMBERS AND GUESTS

OVERVIEW

- PROJECT BACKGROUND
- RECENT DIRECTIONS

INSTITUTE AND INTERNAL AFFAIRS

PAC RECOMMENDATIONS

GROUNDRULES AND ANNOUNCEMENTS

THANKS, AND LET'S HEAR SOME RESULTS

PAC RECOMMENDATIONS

ISSUE	ACTION OR PLAN
ENHANCE PROGRAM REPUTATION	EMPHASIZING PUBLICATION, PRESENTATIONS, AND VISITATIONS
1. EVALUATE AND EXTEND TECHNIQUES FOR INITIATION	THANKS FOR ENCOURAGEMENT; ARE CONTINUING
2. STUDY SIMILARITIES/DIFFERENCES AMONG SPECIES AND TECHNIQUES	RECOGNIZE IMPORTANCE; ARE CONTINUING
CONSIDER MECHANISTIC STUDIES	APPRECIATE ENCOURAGEMENT; CONSIDERING AND DOING
SUBCULTURING = MORE EMBRYOS?	SOME CONFUSION HERE, CAN WE DISCUSS LATER?
3. INITIATION = EASY, HI FREQ; MAINTENANCE/INCREASE = HARD	BELIEVE THE OPPOSITE, AND HAVE PUSHED INITIATION MORE THAN MAINTENANCE/INCREASE
CHEMICAL MAKE-UP OF MUCILAGE	WORKING, BUT SHOULD CONSIDER TESTS OF BIO-ACTIVITY
4. NO NEED FOR MORE BIOCHEM MARKERS, PUT EFFORT ON OTHER SUGGESTED TOPICS	REDEPLOYED EFFORTS, WORKING ON D-FIR, TOTAL REDUCTANTS, PROTEINS, AND OTHER TOPICS
5. DEVELOPMENT/MATURATION AND CONVERSION = KEY ISSUES	LARGEST SINGLE EFFORT, HEAR SOME RESULTS TODAY. EXCITED ABOUT ZYGOTIC/SOMATIC COMPARISONS.
CAUTION ON ROOTS	NO PROBLEMS MEAN LITTLE WORK. IF PROBLEMS, WILL CONSULT.
GROWTH REGULATOR DEBATE	IMPORTANT, PERHAPS HAVE SOMEONE DO FOR US?
6. PROPLASTIDS, INDICATORS OF EMBRYOGENIC POTENTIAL?	FIRST SAMPLES BEING PROCESSED, CONTINUING IN PARALLEL WITH WORK ON MATURE EXPLANTS
NEAR-TERM PLAN:	
FOCUS EXISTING AND NEW TALENT ON REGENERATION	THRUST REMAINS REGENERATION, NEW POST-DOC WILL WORK ON DEVELOPMENT/MATURATION
HOLD GENETIC ENGINEERING	EXPLORATORY RESEARCH

Westvaco

April 27, 1987

Dr. Ronald J. Dinus
The Institute of Paper Chemistry
PO Box 1039
Appleton, WI 54912

Dear Ron:

The Project Advisory Committee meeting of March 30 and 31, 1987 was a success. The prepared materials were appropriate in content, the presentations were clear and well done, and good progress was shown in a variety of pertinent project areas. The only negative note was related to the timeliness of the Progress Report. Several members would like to have had the materials earlier for a more complete review and analysis. Overall, though, the conceptual development as well as the work progress is on target. The emphasis on tissue culture with biochemistry support was demonstrated to be in approximately the right proportions as suggested by previous PAC's, the species emphasis has the right trend, i.e. from the model Norway spruce to the hard pines. Enhancement of the Institute program through publication, presentations, visits, and visiting scientists has progressed well. Plans presented for the next 18 months were in line with Project objectives as viewed by PAC. More specific points follow:

(1) A key accomplishment was the extension of trials of published techniques to achieve embryogenic tissue in a line of Douglas-fir, formation of embryogenic tissue with pond pine, development of embryogenic callus in loblolly pine, and production of embryogenic callus in white pine. These successes indicate that we can most likely achieve embryogenesis for a wide range of species and perhaps with more than one series of techniques. PAC encourages the continuation of these tests.

(2) The studies of embryogenic callus initiation show clear differences in the protocol critical to the initiation of callus with white pine as compared to other species. Optimum embryo development stage, light-dark initiation, hormone levels, genotype, and nutrition levels cause differences in initiation of callus in some species. It is important to continue these types of studies to obtain information on similarities and differences among species and to have available a variety of protocols to meet the variety of industry objectives. Mechanistic studies may be an appropriate means of circumventing bottlenecks in the tissue culture effort when they arise. Again the comment was made that other studies showed more embryos to be produced after several subcultures, a factor that could be considered in similar tests in the future.

Timberlands Division
Forest Research
Box 1950
Summerville, SC 29484
Telephone 803 871 5000

(3) The variety of topics covered in research directed to initiation and maintenance of embryogenic callus in hard pines all appeared to be of an appropriate type to solve the numerous riddles of initiation and maintenance protocol. Basically, the excellent work shows that fairly high frequency initiation of somatic embryo lines can be achieved, but maintenance and buildup is hard to do at this point. Work in tissue culture needs to be concentrated on developing protocols to successfully maintain the white mucilaginous tissue after it has been initiated. It is recommended that the chemical make-up of the mucilaginous material be assessed. It is also recommended that initiation procedures continue to be refined.

(4) The four principal biochemical markers tested during this review period could be used to quantitatively discriminate between embryogenic and non-embryogenic callus. White spruce showed some differing trends. Standard deviations were large in some cases indicating a need for more replications if one were to need higher levels of confidence, but the trends are clear. This work has been extraordinarily valuable in directing research and in predicting responses. There seems, however, to be no current need to develop any other biochemical markers. As indicated by the team, "the four principal biochemical markers (plus two in reserve) when combined with visual, tactile, and ultrastructural observations should be sufficient to evaluate any callus line". Recommendations were: (a) that biochemical marker tests be made on the special Douglas-fir line that sometimes seems embryogenic and sometimes not embryogenic, (b) that the chemical/biochemical basis for the total reductants test be determined and (c) that protein synthesis information be developed to determine how it relates to growth. Decision was reached to defer biochemical tests of genetic fidelity until more plants were available. Overall, the recommendation is to use biochemistry to remove obstacles and explore mechanisms that appear to be keys to embryo development and plant conversion.

(5) Conversion of conifer somatic embryos to plants should be a key research issue at IPC. The combination of talents seems particularly suited for this effort. Proposed work to assess the appropriate level of maturation of the embryo for conversion, to develop techniques to change the rate of maturation of the embryo to improve the current protocol (BSO level, for example), to find the best genetic lines for conversion to plants, and to develop information on the biochemical differences in embryos that convert and those that don't all seem appropriate. Root morphology studies should be approached cautiously to first determine status of work at N.C. State University and in the literature. The question of growth regulators was the subject of some debate without conclusion. Some feel that morphogenic windows are the key to plant conversion and that growth regulators, while they must be present, do not affect conversion in relation to the levels present.

Another suggestion was to assess levels of some growth regulators (perhaps abscisic acid) in natural systems of developing loblolly pine, Norway spruce, and Douglas-fir. These ideas are presented for consideration by the research team.

(6) The ultrastructural examination of embryogenic and non-embryogenic cells in conifers provided insight into chloroplast development. Evidence was presented that showed protoplastids in both zygotic and somatic embryos. The issue was raised as to whether the presence of protoplastids relates to and can be an indicator of embryogenic potential, i.e. could one look for these elements in mature trees and thus find cells which could develop embryos. Research will be directed to screening mature tissues to find protoplastids and test the cell's embryogenic potential.

The plans for the next eighteen months provided in the outline in "PAC Forest Genetics Research Committee Meeting Handouts, March 30-31, 1987" were supported by PAC with a few clarifications, but no significant change. The focus must continue to be on developing a reliable, repeatable process (or processes) for producing plants from cells. Although PAC encourages individual scientists to conduct exploratory research in the area of genetic alteration and engineering, we do not support a new thrust or program focus which expands into those areas. New hires and new research missions should continue to emphasize our objective - plants from cells. Progress and plans of the type presented at this PAC meeting show the way. Some procedure may need to be developed to chart the progress along the way, to show when the research team has achieved a "landmark" with the several species being studied. All species are not at the same developmental stage toward a reliable plant production system and it is difficult for PAC to know the status of each of the several species of interest. Please give such a system some thought.

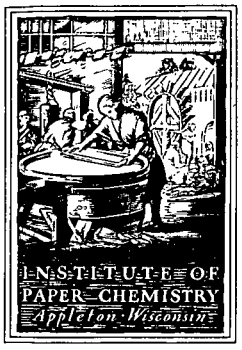
Overall, the meeting was a good one. We thank you and the team for their thorough preparation and presentations and for working so hard to provide the progress in this program.

Very truly yours,

Ed Owens

Edwin G. Owens
Westvaco Corporation
Forest Research Director and
PAC Chairman, Forest Genetics

EGO:eds
cc: Forest Genetics PAC Members



THE INSTITUTE OF PAPER CHEMISTRY
Post Office Box 1039
Appleton, Wisconsin 54912
Phone: 414/734-9251
Telex: 469289

September 8, 1987

TO: Chairman and members of the Forest Genetics Project Advisory Committee

SUBJECT: Summary of Recent Activities, Response to Suggestions, and Meeting Alert

Dear Ed and PAC Members:

This is to inform you of recent activities, respond to comments about our last meeting, and to remind all of the next meeting. We acted on many of your suggestions and appreciate the kind compliments. Thanks also for advice and help given over the summer.

Before dealing with technical issues and our October meeting, I must mention a number of other happenings. New RAC and PAC members have been selected. Changes in RAC composition should make the group more supportive of our work. Joining us as new PAC members are Sharon Miller, Chesapeake; James Schuler, Green Bay Packaging; and Herschel Webb, Kimberly-Clark. Ron Woessner is returning for another term; thanks to Mead for allowing us to retain his services. We look forward to working with these new folks and with the committee as a whole.

On other fronts, we have all been active on the meeting circuit, with participation as good as or better than in earlier years. Russ Feirer completed his preliminary examinations with flying colors and is now on the last leg of degree completion. In the midst of all that, he also participated in several conferences, including involvement as an invited speaker at the annual meeting of the National Forest Products Research Society. We are likewise holding our own or improving on the publication front, and involvement as reviewers of manuscripts and grant proposals has also increased. Visitors have been several and most helpful. I firmly believe that increased effort on these fronts has significantly sharpened our image and enhanced our reputation.

With regard to staffing, the administration has provided funding for a Post Doctorate. Our intention is to have the individual on board before the first Industrial Research Fellow departs, and to focus this new talent on embryo development/maturation. We continue to seek commitments for additional Research Fellows or Post Doctorates. Several leads have been developed, and we are working hard to solidify arrangements with one member company. Also, Georgia-Pacific has joined the Institute, and seems quite interested in cloning and related research. Prospects for bringing new students into the project look promising.

Work over the last several months has proceeded generally in accordance with the plan presented last spring, and with suggestions surfaced during the meeting or in your follow-up letter. Given the many points of agreement, our response is rather brief.

In keeping with our mutual understanding, we continue to emphasize tissue culture work, to utilize biochemistry in support of it, and to extend findings from the model system to our target species. Responses to your more specific points are given below, arrayed in accordance with the numbers used in your letter.

1. Thanks for the encouragement, and we continue to test these and newer techniques.

2. Species similarities and differences are important, and such studies are being continued.

   We recognize existence and persistence of "bottlenecks" on several fronts, and are moving to break or circumvent them. Studies include more "mechanistic" approaches as well as the varying of nutritional and growth regulator regimes. Your encouraging us to undertake more of the former is appreciated.

   The comment about subculturing has somewhat confused us. All things considered, I suggest that we discuss this issue in greater depth at our next meeting.

3. Initiation vs. Maintenance/Build-Up. Regrettably, results are opposite perception. Initiation frequencies in target species are low, whereas the few lines forming white mucilaginous callus are rather easily maintained and increased. Little reversion is noted. Both aspects need work, but we are pushing initiation harder.

   The mucilaginous material continues to intrigue us, and some work is being done on its chemical nature. Before expending great effort, however, we are considering testing its biological activity. Will adding it or extracts of it affect growth or development?

   As noted above, we are refining initiation protocols.

4. Efforts formerly devoted to developing additional markers are now deployed on other fronts, including tests of the "on again/off again" Douglas-fir and basis of the total reductants test. Some work on protein synthesis and its relationship to growth is under way. As inferred in Item No. 2 above, we are initiating biochemical studies of mechanisms underlying embryo development.

5. Agreed, development/maturation of somatic embryos, and subsequent conversion to seedlings should be a key research issue. Our plan called for increased activity in this area, and we have much work under way. Your suggestions were considered in choosing approaches and designing studies.

   Root development has not proved a problem in the few seedlings produced to date. Should problems surface, we would be certain to consult available authorities.

   The team believes that understanding growth regulator levels, and changes therein, is important, but that a major commitment is unwise at the present time. Developing an understanding would require much effort, thereby detracting from other key activities. We, however, are exploring opportunities for cooperative investigations, particularly of abscissic acid relationships, with workers elsewhere.

6. A number of mature tissues are being considered as explant sources, and samples have been isolated and prepared for microscopic examination.

Concerning your closing comments, the main thrust of existing and new personnel will remain regeneration of plantable seedlings from one or a few cells. Work on gene transfer will be continued at a low level, on an exploratory basis and largely by students. We cannot afford to lose complete touch with advances in this area. Thought is being given to systems for portraying degree of progress vs. intensity of effort by developmental stage and species. If designed well, such a system should enable PAC to better understand our status/needs as well as provide us with another means for mid-course correction.

As mentioned at our last meeting, the President has requested a long-range (three to five year) plan for each Division at IPC. Acting in concert with the other Directors, we prepared a preliminary proposal and shared it with the administration and RAC last week.

Assumptions used in the planning process were several. As examples, we were asked to assume a doubling of resources over the five-year period as a result of (1) more funding from increased numbers of IPC members and (2) heightened abilities to tap external sources. We were further asked to project what we thought it best to be doing five years hence, and how we might best grow to do it.

In preparing our contribution, we relied first and most heavily on the 12 to 18 month plan shared with you last March. Also helpful was the report derived from the Forest Biotechnology Workshop held in Raleigh last January. Several companies active on our PAC were represented in depth at that workshop, and we view the document as a fair rendering of industry needs and wants. In addition, I was able to discuss present and future needs with leaders from member companies not now represented on PAC but becoming more interested in mass cloning, other applications of tissue culture, and genetic engineering.

Since the plans will be discussed again with RAC at their next meeting on September 24-25, I would appreciate your studying the enclosed copy and communicating any comments or questions to me or your RAC representative. In studying the plan, please note that we have ensured continued emphasis on somatic embryogenesis and regeneration. In fact, the proposal specifies an increase in resources for the present effort, via reshuffling of existing personnel and addition of new and different talent. This seems quite in accord with our mutual understanding. "New" projects aimed at emerging needs are suggested, but only after needs for refinement and expansion of ongoing work are met.

Lastly, please note on your calendars that our next meeting is scheduled for October 26-27 here in Appleton. We are planning to present our work during the first afternoon, and have the usual committee session the following morning. Invitations and an agenda will be forwarded in the near future.

Many thanks, and we look forward to visiting with you in October.

Sincerely,

Ron/sb

Ronald J. Dinus
Director
Forest Biology Division

RJD/sjb
Enclosures

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

Status Report

to the

FOREST GENETICS

PROJECT ADVISORY COMMITTEE

Project 3223

THE MASS PRODUCTION OF CONIFERS

October 26-27, 1987

PROJECT SUMMARY FORM

DATE: September 28, 1987

PROJECT NO. 3223 - Mass Production of Conifers

PROJECT STAFF: M. Becwar*, R. Dinus, R. Feirer, M. Johnson, R. Nagmani, S. Verhagen, S. Wann*

PROJECT OBJECTIVE/GOAL:

Overall - Mass production of genetically improved conifers.

Near-term - Develop procedures for producing plantlets from single cells or small groups of cells.

PROJECT RATIONALE:

Major increases can be obtained in tree growth, quality, and uniformity via rapid, low-cost methods for mass cloning of "elite" trees. Reliable cell and tissue culture systems will also open the way for genetic engineering, and production of new genetic combinations having exceptional growth, increased pest resistance, special fiber qualities, and enhanced site and/or climatic adaptability. Screening for and selecting useful variants in culture could also lower costs and raise efficiency of conventional tree breeding. Cell and tissue culture thus form the basis for second-generation technologies, which could improve upon existing technologies or greatly accelerate their pace.

CURRENT FISCAL YEAR BUDGET: $600,000

SUMMARY OF RESULTS SINCE LAST REPORT: (March 1987 - September 1987)

Methods previously developed for initiating callus, growing cells in suspension, and maintaining callus and cell lines remain in use or are being refined. Results from past work on nonconifer model systems, natural embryo

*Industrial Research Fellow

SUMMARY OF RESULTS SINCE LAST REPORT (Contd.)

development, and organogenesis have been integrated and are being applied to the Norway spruce model system and target species. The most reliable biochemical and histological markers are being used to screen cultures for embryogenic potential, and monitor effects of modified or new protocols.

Embryogenesis in the model system is now controlled and reproducible. Initiation of embryogenic callus from both immature and mature embryos is straightforward; embryo numbers can be quantified and seedlings recovered. Increased embryo numbers and development have been noted in suspension cultures, and good yields of viable protoplasts have been obtained. Experiments with the model system now use mature embryos, thus permitting year-round work. Similar progress has been made with white spruce, a commercially important species.

Though seedlings have been recovered, development/maturation of spruce somatic embryos remains difficult, and increasing efficiency of these steps remains a major effort. Results from earlier work on biochemistry of nonconifer model systems are proving helpful. Also, use of abcisic acid in development media has given positive results. Such refinements show potential for increasing seedling recovery, and results are being extended to target species as quickly as experiments are completed. Spruce seedlings recovered earlier entered dormancy and renewed vegetative growth in synchrony with their zygotic counterparts.

Histological assays have shown that spruce embryogenic callus originates from the few outermost cell layers of the hypocotyl. Accordingly, work with more mature spruce materials is using tissues similar to those in hypocotyls.

Somatic embryogenesis was obtained in white pine some months ago. The callus is similar to that of spruces, but origin and factors promoting formation are different. In white pine, embryogenic callus forms from the suspensor region, rather than from the hypocotyl as in spruce. In addition, initiation frequencies appear to vary with donor tree, suggesting genetic differences in embryogenic potential. Progress on development/maturation has been slow but positive. Lessons from the white pine experience are facilitating work on target species.

Embryogenic callus has also formed in cultures of pond and loblolly pines. Initiation frequencies have been low and variable. Even so, callus has been obtained from a variety of sources, including winter collections from South America and summer collections from the southeastern U.S. All lines are phenotypically and biochemically similar to spruce embryogenic callus. More recently, initiation was extended to pitch x loblolly pine hybrids. The method is thus reproducible, and appears generally applicable to pine species. Current efforts are focused on increase and development/maturation.

Status of Douglas-fir remains much the same. Embryogenic callus can be initiated, but not at will. Initiation frequencies are low. Increase is difficult and development/maturation has not been achieved. Experiments will continue over the winter months with stored cones and collections from New Zealand.

Biochemical/molecular tests of differences between embryogenic and nonembryogenic callus continued. A low molecular weight protein was found in most nonembryogenic calli, but was absent from comparable embryogenic cultures. The protein apparently is associated with differences in chloroplast development described earlier. In addition, techniques for isolating, purifying, and

characterizing both RNA and DNA have been developed or refined for use in assessing similarities/differences between embryogenic and nonembryogenic calli, immature and mature explants, and somatic and zygotic seedlings.

Work, on all fronts, over the last six months has proceeded and will continue for the immediate future in accordance with the plan approved last spring.

SHORT TERM GOALS:

Goals for Remainder of FY 87-88

1. Refine protocols for initiating embryogenic callus in target species. Exploit results from latest work on explant development stages and capitalize on findings from other laboratories. Characterize and compare cultures, using best biochemical markers and analyses.

2. Accumulate baseline data on histological, biochemical, and molecular characteristics of somatic and zygotic embryogenesis. Use biochemical markers, related biochemical analyses, and molecular tools to identify embryogenic potential, facilitate initiation of embryogenic callus, and improve development/maturation.

3. Improve protocols for increasing development/maturation frequencies, raising efficiency of conversion to seedlings, and providing sufficient material for replicated greenhouse trials. Develop and test biochemical and molecular tools for evaluation of fidelity and performance.

4. Increase ability to obtain and mature somatic embryos in alternative culture systems. Exploit recent progress with suspension cultures. Concentrate on systems well-suited to mass propagation.

SHORT TERM GOALS (Contd.)

5. Continue work on protocols for obtaining embryogenic callus from more mature explants. Determine culture factors and explant characteristics that must be altered, and evaluate procedures for changing them.

6. Execute exploratory research on origin of embryogenic callus, regeneration from single cells or protoplasts, promising molecular techniques, and genetic transformation.

7. Secure additional loblolly pine and Douglas-fir explant sources from southern hemisphere. Maintain potential for year-round experimentation.

8. Provide for prompt publication of findings.

COOPERATIVE INVESTIGATIONS:

1. North Carolina State University - Cooperative evaluation with Dr. R. Mott and Dr. H. Amerson of procedures for initiating embryogenic callus cultures of loblolly pine, Norway spruce, and white spruce.

2. Ohio State University, Wooster - Dr. Kriebel supplied immature white pine cones for initiation trials, and assisted in preparation of two manuscripts.

3. Williams College/Merrell-Dow Pharmaceutical Co. - Cooperative study with Dr. Robert Slocum (Williams) plus Drs. A. Bitonti and P. McCann (Merrell-Dow) of polyamine metabolism, and joint preparation of resultant manuscript.

4. International Forest Seed Company - Supply of "rejuvenated" loblolly pine material by Dr. S. Foster for experiments on initiation of embryogenic callus from mature explants.

5. University of Cincinnati - Joint assay with Dr. J. Caruso of endogenous hormone levels, principally ABA and IAA, in embryogenic and nonembryogenic calli. Arrangements now being finalized.

RELATED STUDENT RESEARCH:

Completed in 1987

Tyrone Cornbower - M.S., Independent Study, entitled "Response of white spruce to mechanical pulping following hemicellulose hydrolysis."

Luke Nealey - Ph.D. Program, Organic Chemistry Orientation, entitled "Isolation and characterization of xyloglucan from suspension cultured loblolly pine cell medium."

In Progress

Michael Bogenschutz - M.S., Independent Study, entitled "Electroporation-mediated genetic transformation of Norway spruce cells."

Russ Feirer - Ph.D. Program, Biochemical Orientation, entitled "Biochemical and molecular studies of plant development." In cooperation with University of Wisconsin, Madison.

Note: Several students in the class entering this fall, have expressed an interest in Project 3223.

INITIATION AND MAINTENANCE OF EMBRYOGENIC CALLUS

MICHAEL R. BECWAR

INITIATION AND MAINTENANCE OF EMBRYOGENIC CALLUS FROM IMMATURE EXPLANTS IN PINES

TOPICS

1. CENSUS OF CULTURES
2. EXPLANTS, PROTOCOLS EVALUATED, AND MEDIA
3. INITIATION FROM BRAZILIAN LOBLOLLY PINE
4. INITIATION DURING SUMMER 87, LOBLOLLY AND PITCH X LOBLOLLY PINE
5. CONCLUSIONS - THE OPTIMUM INITIATION PROTOCOL

CENSUS OF EXPLANTS CULTURED AND
INITIATION OF EMBRYOGENIC CALLUS (EC) IN PINES

PINE SPECIES	EXPLANT COLLECTION SITE[a]	TIME	NUMBER OF EXPLANTS	EC LINES
POND	GA	JULY, 86	5,053	2
EASTERN WHITE	WI & OH	JULY, 86	2,726	8
LOBLOLLY	GA & SC	JULY, 86	1,775	9
	BRAZIL	JAN., 87	8,991	6
	GA & SC	JULY, 87	15,667	45
PITCH X LOBLOLLY	SC	JUNE, 87	885	6
		TOTALS:	35,097	76

[a]GA = RINCON, GEORGIA; WI = GREENVILLE, WISCONSIN;
OH = WOOSTER, OHIO; SC = SUMMERVILLE, SOUTH CAROLINA;
AND BRAZIL = SANTA CATARINA, TRES BARRAS.

EXPLANTS AND PROTOCOLS

1. POST-FERTILIZATION FEMALE GAMETOPHYTE
   - SMITH, 85
   - IPC, 86
   - GUPTA AND DURZAN, 87

2. IMMATURE EMBRYOS
   - GUPTA AND DURZAN (SUGAR PINE), 86
   - IPC (WHITE PINE), 86

DIFFERENCES IN NITROGEN
COMPONENTS AND ADDENDA OF BASAL MEDIA

BASAL MEDIA CODE	REFERENCE	NITROGEN (mM) NH_4^+	NO_3^-	GLUTAMINE	ADDENDA[a]
MS	MURASHIGE & SKOOG, 62	21	39	0	---
MSG	AMERSON ET AL., 85	0	1	10	---
MSCG	IPC, 86	0	1	4	+CH
DCR	GUPTA & DURZAN, 86	5	13	2	+ INOSITOL [2 X MS] +CH
DZL	GUPTA & DURZAN, 87	7	53	3	+ INOSITOL [10 X MS] +CH

[a]CH = CASEIN HYDROLYSATE

BRAZILIAN LOBLOLLY
PINE CONES

- HARVESTED FROM PROGENY
TREES OF SIX FAMILIES

SUMMARY OF EMBRYOGENIC CALLUS (EC)
ESTABLISHED FROM BRAZILIAN LOBLOLLY
PINE WHOLE FEMALE GAMETOPHYTE EXPLANTS

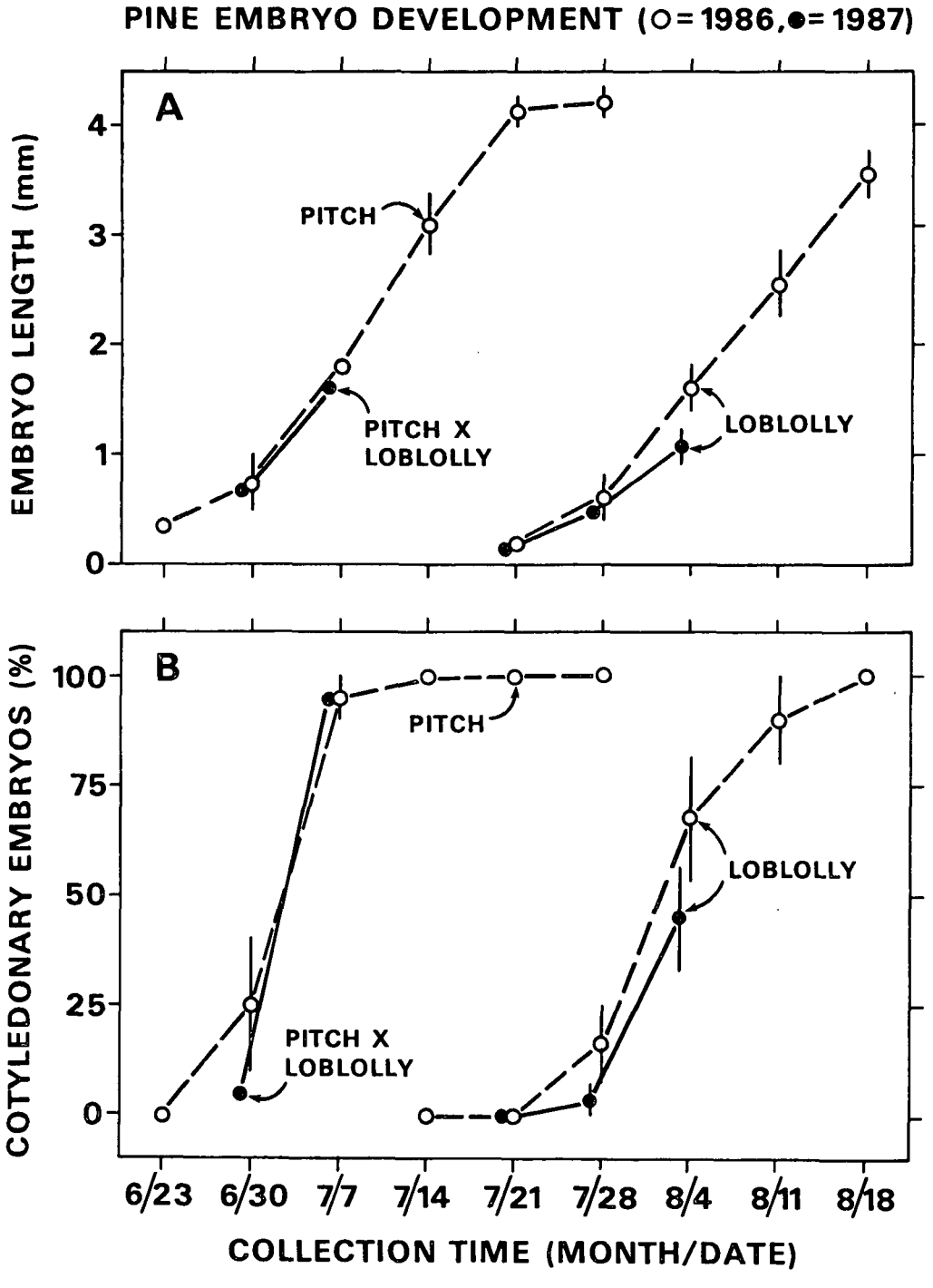
INITIATION MEDIUM	EXPERIMENTS[a]	NUMBER OF EXPLANTS	NUMBER OF EC LINES
MSG 0/0	I, II, & III	2,500	3
DZL 11/4,5	III & IV	1,528	0
MSG 2/1	IV	1,040	0

[a]I = RP537, II = RP543, III = RP545, and IV = RP555

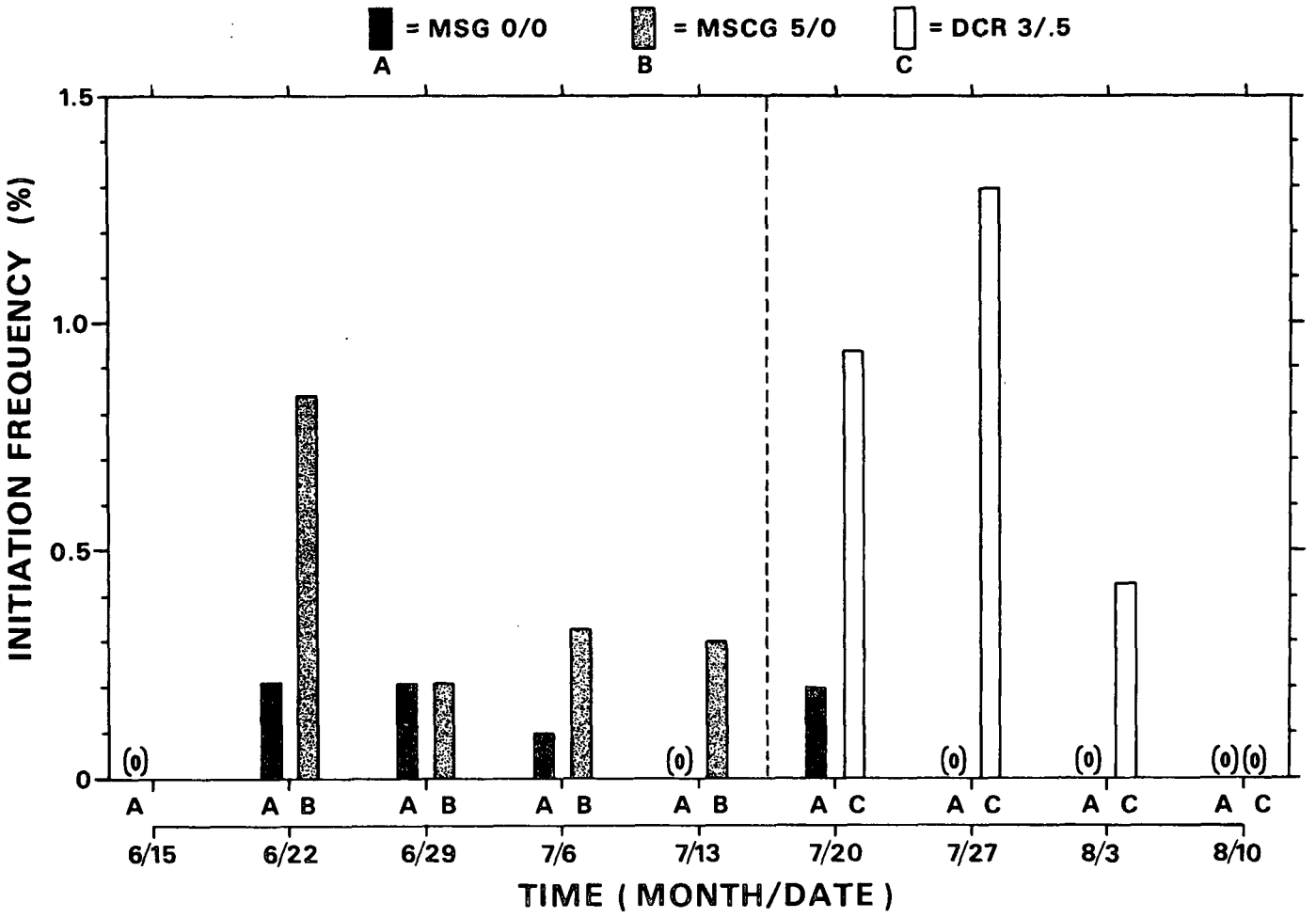
SUMMARY OF EMBRYOGENIC CALLUS ESTABLISHED
FROM BRAZILIAN LOBLOLLY PINE EMBRYO EXPLANTS

EMBRYO EXPLANT		NUMBER OF EC LINES[a]		
COLLECTION DATE	DEVELOPMENTAL STAGE	MEDIUM MSG 2/1	DCR 3/.5	MSCG 5/0
2/3/87	PRECOT.	0	0	2
2/10/87	MOSTLY PRECOT.	0	0	1

[a]400 TO 500 EXPLANTS FROM FIVE SEED FAMILIES CULTURED PER EACH TREATMENT AND DATE.



Time course of embryo development in loblolly, pitch, and pitch x loblolly pine in 1986 (open circles) and 1987 (closed circles) during the time of culture initiation. A: Embryo length. B: Percentage of embryos with cotyledons.

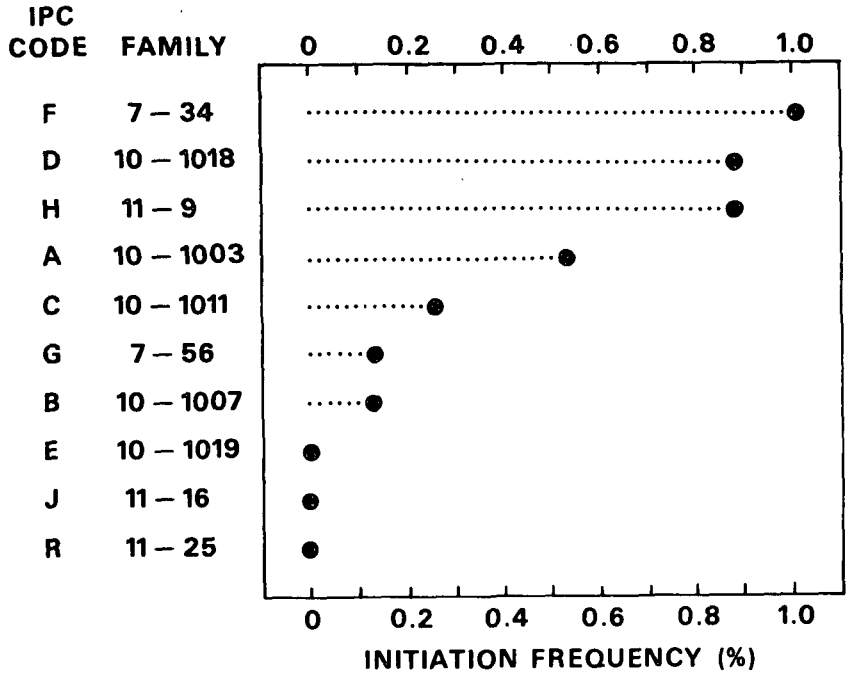


The initiation frequency of embryogenic callus as a function of time in loblolly pine using three protocols. A: Whole female gametophyte explants on MSG 0/0 (solid bars). B: Whole female gametophyte explants on MSCG 5/0 (shaded bars). C: Immature embryo explants on DCR 3/.5.

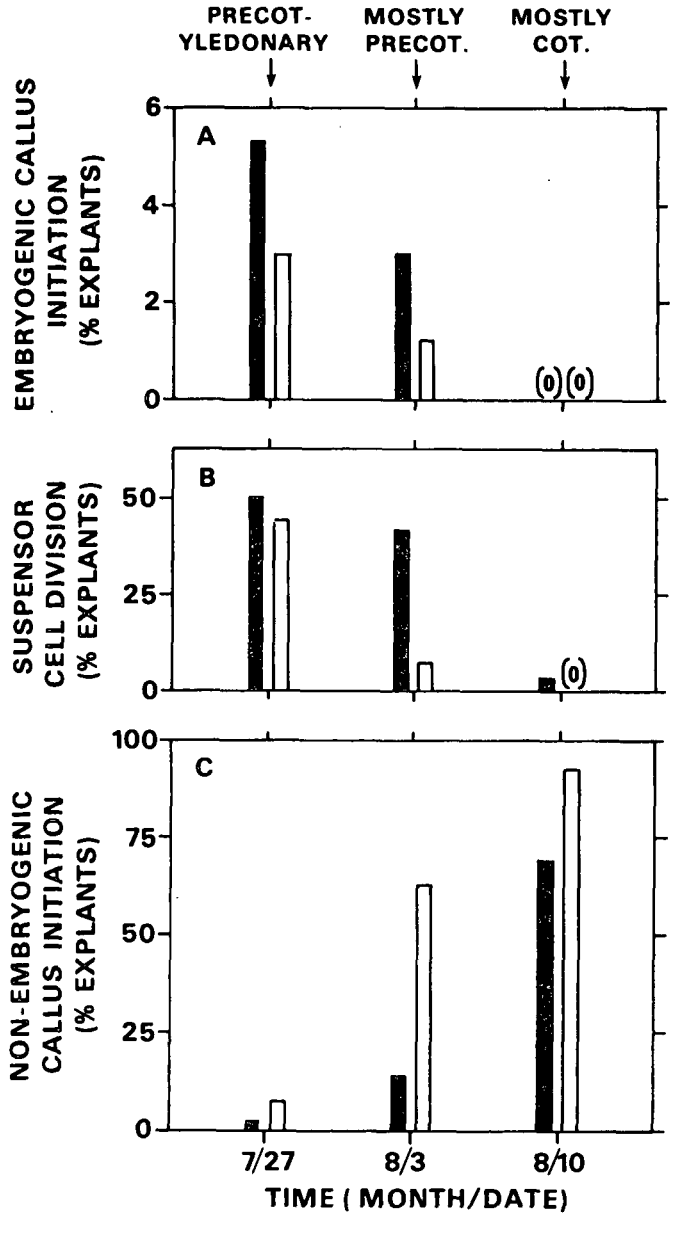
COMPARISON OF THE FREQUENCY OF INITIATION OF EMBRYOGENIC CALLUS IN LOBLOLLY PINE ON THE SMITH (MSG 0/0) AND THE GUPTA DURZAN (DZL 11/4,5) PROTOCOLS

	INITIATION FREQUENCY, %[a]			
	CLONE F		CLONE H	
	MEDIUM		MEDIUM	
(MONTH/DATE)	MSG 0/0	DZL 11/4,5	MSG 0/0	DZL 11/4,5
6/15	0	0	0	0
6/22	0	0	0.9	0
6/29	0	0	0.9	0
7/6	0	0	0	0
7/13	0.8	0	0	0
7/20	0.8	0	0	0
7/27	0	0	0	0
8/3	0	0	0	0
8/10	0	0	0	0

[a]POST-FERTILIZATION FEMALE GAMETOPHYTE EXPLANTS, 75 TO 120, CULTURED PER EACH CLONE, MEDIUM TREATMENT, AND DATE.



Comparison of initiation frequency of embryogenic callus among ten families of loblolly pine. Each datum point includes initiation from three protocols, MSG 0/0 (Smith), MSCG 5/0 (IPC), and DCR 3/.5 (White pine). A total of 1000 explants cultured per family.



The time course of three morphogenic responses of immature embryos of loblolly pine cultured on DCR 3/.5 medium. A: Initiation frequency of embryogenic callus. B: Frequency of suspensor cell division. C: Initiation frequency of nonembryogenic callus. The stage of explant development is shown on the top. Solid bars = clone F (7-34) and open bars = clone H (11-9).

INITIATION OF EMBRYOGENIC CALLUS (EC) IN PITCH PINE X LOBLOLLY PINE HYBRID

EXPLANT	COLLECTION (MONTH/DATE)	EC INITIATION FREQUENCY, %[a] MEDIUM			
		MSG 0/0	MSG 2/1	DCR 3/.5	MSCG 5/0
POST-FERTILIZATION F. GAMETOPHYTE	6/8	0	--	--	--
	6/15	0.9	--	--	--
	6/22	0	--	--	--
EMBRYO	6/29	--	1.1	2.1	0
	7/6	--	1.0	0	1.0

[a]85 TO 110 EXPLANTS CULTURED PER TREATMENT.

SUMMARY

1. OPTIMUM INITIATION WINDOW IN PINUS IS DURING PRECOTYLEDONARY EMBRYO DEVELOPMENT.

2. THE PRECISE TIME OF THE WINDOW VARIES WITH THE EXPLANT AND PROTOCOL USED.

3. IN LOBLOLLY PINE THE OPTIMUM INITIATION PROTOCOL WAS DARK CULTURE OF PRECOTYLEDONARY EMBRYOS (LESS THAN 0.5 MM IN LENGTH) ON DCR 3/.5.

4. WE HAVE NOT BEEN ABLE TO INITIATE VIA THE LOBLOLLY PINE PROTOCOL PUBLISHED BY GUPTA AND DURZAN.

5. ONE LOBLOLLY PINE EMBRYOGENIC LINE APPEARED SIMILAR TO SPRUCE, IN THAT, NUMEROUS SOMATIC EMBRYOS COVERED THE SURFACE OF THE CALLUS.

6. VARIATION AMONG EMBRYOGENIC LINES IN THE FREQUENCY AND DEVELOPMENTAL CAPACITY OF SOMATIC EMBRYOS EMPHASIZES THE IMPORTANCE OF IMPROVING INITIATION FREQUENCY TO FIND HIGHLY EMBRYOGENIC LINES.

7. THE HIGHEST INITIATION FREQUENCY IN LOBLOLLY PINE, 5%, RESULTED FROM PERSISTENT DIVISION OF CELLS IN THE SUSPENSOR REGION. OBSERVATIONS ON INITIATION OF SUSPENSOR CELL DIVISION SUGGEST A POTENTIAL FOR SIGNIFICANTLY IMPROVING INITIATION FREQUENCIES.

8. EMBRYOGENIC CALLUS HAS BEEN INITIATED IN PITCH X LOBLOLLY PINE HYBRID.

DOUGLAS-FIR AND MATURE EXPLANTS

NAMANI RANGASWAMY

ATTEMPTS TO INDUCE EMBRYOGENIC
CALLUS FROM DOUGLAS-FIR EMBRYOS

EXPERIMENTAL PROTOCOL FOR INITIATION OF EMBRYOGENIC CALLUS

COLLECTION DATES	EXPLANT SOURCE	MEDIUM + SUPPLEMENTS	ENVIRONMENT
7/7/87	IMMATURE EMBRYO (PRECOTYLEDONARY)	HM 2 2,4-D/1 BA	BOTH LIGHT & DARK
7/17/87	(COTYLEDONARY STAGE)	MSCG 5 2,4-D/0 BA	
7/23/87	" "	DCR 3 2,4-D/0.5 BA	
7/30/87	" "	MSG 2 2,4-D/1 BA	
8/6/87	" "	1/2 BLG 2 2,4-D/0.2 BA	
8/12/87	" "	1/2 BLG 2 NAA/1 BA	

IS THE RESPONSE OF CULTURED DOUGLAS-FIR EMBRYOS TO DIFFERENT TREATMENTS SIMILAR TO THAT OF MODEL SPECIES (SPRUCES) OR THE OTHER TARGET SPECIES (PINES)?

COMPARISON OF DOUGLAS-FIR WITH SPRUCES AND PINES

	REPRODUCTIVE CYCLE, YR	EXPLANT	RESPONSE TO MEDIA AND TREATMENTS
SPRUCES	1	IMMATURE EMBRYO AT COTYLEDONARY STAGE	FORMATION OF EMBRYOGENIC CALLUS IN LIGHT; IN ASSOCIATION WITH GREEN, NONEMBRYOGENIC CALLUS
PINES	2	IMMATURE EMBRYO AT PRECOTYLEDONARY STAGE	FORMATION OF WHITE EMBRYOGENIC CALLUS ONLY IN DARK; EXTRUDED CALLUS FROM OVULES ALSO
DOUGLAS-FIR	1	IMMATURE EMBRYOS PRECOTYLEDONARY COTYLEDONARY	FORMATION OF WHITE CALLUS IN BOTH LIGHT AND DARK, BUT SOME CALLUS LINES TURN GREEN ON TRANSFER TO LIGHT

SUMMARY

1. OBTAINED WHITE, MUCILAGINOUS CALLUS (EMBRYOGENIC?) FROM EXPLANTS AT HIGHER FREQUENCY.

2. CALLUS AND CELL TYPES PHENOTYPICALLY RESEMBLE EMBRYOGENIC SPRUCE CALLUS.

3. GLOSSY MUCILAGINOUS CALLUS

   EXPLANT (PRECOTYLEDONARY)

   - ORIGINATES FROM SUSPENSORS
   - CANNOT BE MAINTAINED

   EXPLANT (COTYLEDONARY)

   - ORIGINATES FROM HYPOCOTYL
   - CAN BE MAINTAINED (SOME CALLUS LINES)

FUTURE RESEARCH PLANS DESIGNED TO INDUCE SOMATIC EMBRYOGENESIS IN DOUGLAS-FIR

1. CULTURE EMBRYO EXPLANTS FROM NEW ZEALAND CONE COLLECTIONS (FOR DIFFERENT GENETIC SOURCES).
2. TEST DIFFERENT PROTOCOLS FOR INITIATION OF EMBRYOGENIC CALLUS.
3. CULTURE EMBRYOS FROM MATURE SEEDS.

ATTEMPTS TO INDUCE EMBRYOGENIC CALLUS FROM "MATURE" EXPLANTS

CHOICE OF "MATURE" EXPLANTS

1. SELECT THE MOST JUVENILE TISSUES WITHIN THE TREE.
2. REJUVENATE PARTS OF THE DONOR TREE BY SPECIAL TREATMENTS.

EXPERIMENTAL PROTOCOL USED FOR INITIATION OF EMBRYOGENIC CALLUS FROM "MATURE EXPLANT"

NATURE OF EXPLANT	SPECIES	MEDIA	RESPONSE
VEGETATIVE BUDS	NORWAY SPRUCE WHITE SPRUCE (25-YR OLD TREES)	HM + 2 2,4-D/1 BA 1/2 BLG + 2 NAA/1 BA	FORMATION OF WHITE, GRANULAR CALLUS, WHEN INITIATED IN DARKNESS; TURNED YELLOW LATER.
POLLEN CONES BEFORE MEIOSIS	WHITE SPRUCE	MSG 2 2,4-D/1 BA DCR 3 2,4-D/0.15 BA	" " "
SEED CONES BEFORE MEIOSIS	NORWAY SPRUCE WHITE PINE (40-YR OLD TREES)	MSCG 5 2,4-D/0 BA	" " "

CURRENT ATTEMPTS TO INDUCE EMBRYOGENIC CALLUS
FROM RUST-RESISTANT VARIETY OF LOBLOLLY PINE
(3-YR OLD HEDGE MATERIAL)

NUMBER OF FAMILIES	NUMBER OF CLONES	EXPLANT	MEDIUM + SUPPLEMENTS	RESPONSE
4	16	PRIMARY NEEDLE	1/2 - BLG 2% SUCROSE 2 NAA/1 BA	CALLUS FORMATION
			DCR - 3 2,4-D/0.5 BA	" "
		STEM SECTIONS	MSG - 2 2,4-D/1 BA	" "
		NEEDLES/ FASCICLES	MSCG - 5 2,4-D/0	" "
			1/2 MSG - 5 BA/5K	" "

FUTURE RESEARCH PLANS DESIGNED
TO INDUCE SOMATIC EMBRYOGENESIS

1. TRANSFER CALLUS LINES OBTAINED TO MEDIA FOR SHOOT BUD INDUCTION.
2. CULTURE THESE SHOOTS BUDS ON EMBRYO INDUCTION MEDIA.
3. OBTAIN CELL SUSPENSIONS FROM CALLUS LINES AND USE LIQUID SUSPENSION CULTURES FOR INDUCTION OF SOMATIC EMBRYOGENESIS.

EMBRYO DEVELOPMENT AND MATURATION

SOMATIC EMBRYOS

WHITE SPRUCE, IMMATURE AND MATURE

JUDY WYCKOFF

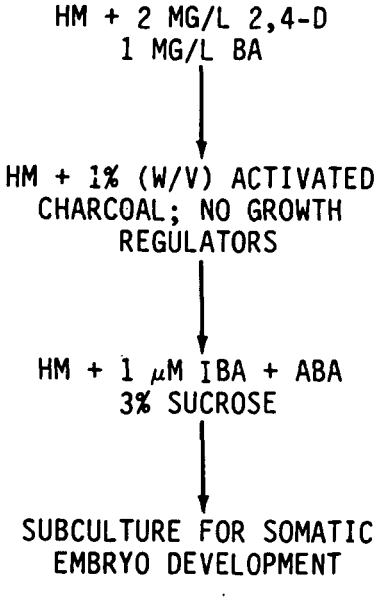
SOMATIC EMBRYO DEVELOPMENT

WHITE SPRUCE

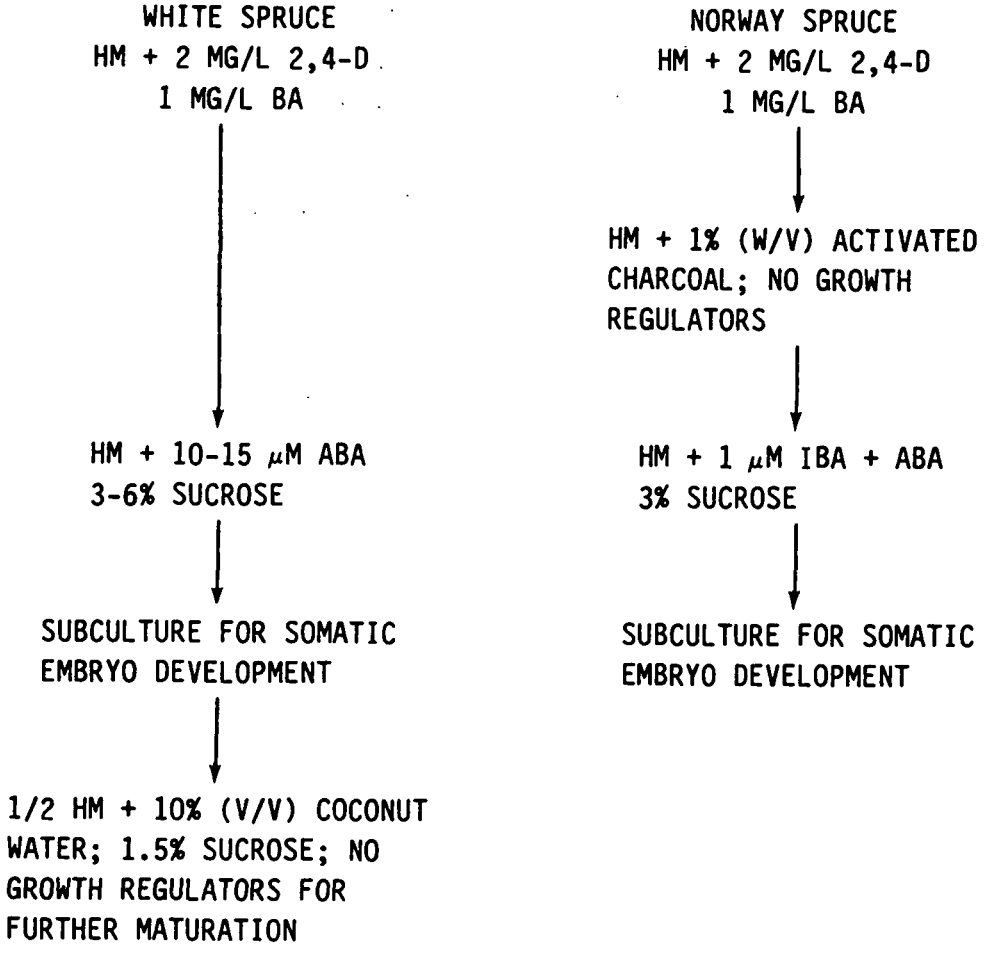
OBJECTIVES

1. EXAMINE FACTORS WHICH THWART SOMATIC EMBRYO DEVELOPMENT IN WHITE SPRUCE.
2. INITIATE EMBRYOGENIC CALLUS FROM MATURE SEED TO PROVIDE AN UNRESTRICTED SOURCE OF EXPLANT MATERIAL CAPABLE OF PRODUCING SOMATIC EMBRYOS.
3. DEVELOP A CONIFER MODEL SYSTEM WITH GOOD CONTROL OVER THE GENETIC BASE.

NORWAY SPRUCE PROTOCOL



COMPARISON OF DEVELOPMENT PROTOCOLS



SUMMARY

1. EMBRYOGENIC CALLUS HAS BEEN INDUCED FROM MATURE SEED OF WHITE SPRUCE AND HAS PRODUCED SOMATIC EMBRYOS.

2. INCREASED ABA LEVELS, SUCROSE LEVELS, AND THE ADDITION OF COCONUT WATER ARE IMPORTANT FACTORS IN THE INDUCTION AND DEVELOPMENT OF WHITE SPRUCE SOMATIC EMBRYOS.

3. RESULTS ARE BEING TESTED ON THE DEVELOPMENT OF SOMATIC EMBRYOS IN EMBRYOGENIC PINE CALLUS.

EMBRYO DEVELOPMENT AND MATURATION

SOMATIC EMBRYOS

LOBLOLLY PINE, IMMATURE

LYNN KROLL

LOBLOLLY PINE SOMATIC EMBRYO DEVELOPMENT

OBJECTIVE

TO PRODUCE SOMATIC EMBRYOS FROM LOBLOLLY PINE CALLUS CULTURES.

LOBLOLLY PINE SOMATIC EMBRYO DEVELOPMENT PROTOCOL

DCR BASAL MEDIUM
+ 1.4 x 10^{-5}M 2,4-D
+ 2.2 x 10^{-6}M BA
14 DAY SUBCULTURE, DARK

↓

MSG BASAL MEDIUM
+ 10 μM ABA + 6% SUCROSE
4-5 WEEKS, DIM LIGHT

EFFECT OF ABA AND SUCROSE ON
SOMATIC EMBRYO DEVELOPMENT

% SUCROSE	ABA (μM) 0	2	5	10	15
3	0	0	0	1-3	0
6	0	0	1-3	7-10	1-3

NUMBER OF SOMATIC
EMBRYOS OBSERVED PER CALLUS

LOBLOLLY PINE EMBRYOGENIC CALLUS LINES

CODE		SOMATIC EMBRYOS OBSERVED
(488 LP 13 B)1	IMMATURE EMBRYO	
(501 LP 12 F)1	COLD STORED IMMATURE EMBRYO	X
(501 LP 12 F)2	" " " "	
(501 LP 12 F)3	" " " "	
(501 LP 13 F)5	" " " "	X
(501 LP 13 F)6	" " " "	X
(506 LP 11 H)20	OVULE/EMBRYO EXTRUSION	
(506 LP 11 H)21	" " "	
(524 LP 12 H)1	COLD STORED IMMATURE EMBRYO	X
(537 LP 1 L)1	IMMATURE EMBRYO	
(537 LP 3 L)1	" "	
(537 LP 1 K)2	" "	X
(537 LP 2 N)3	" "	
(537 LP 2 N)4	" "	X
(543 LP 1 O)1	OVULE/EMBRYO EXTRUSION	

SUMMARY

1. ELEVATED ABA AND SUCROSE LEVELS ARE IMPORTANT FOR LOBLOLLY PINE SOMATIC EMBRYO DEVELOPMENT.

2. GENOTYPE OF TISSUE IS MOST IMPORTANT. ONLY ONE OUT OF NINE LINES TESTED DEVELOPES SOMATIC EMBRYOS REPRODUCIBLY.

   A. ALL NINE LINES HAVE DIFFERENT LOOKING CALLUS.

   B. NO SOMATIC EMBRYOS HAVE DEVELOPED FROM LINES INITIATED UNDER THE SMITH PROTOCOL.

3. TIMING OF SOMATIC EMBRYO DEVELOPMENT IN CALLUS IS CRITICAL.

ZYGOTIC EMBRYOS, LOBLOLLY PINE

STEVE WANN

EFFECTS OF THE IN VITRO ENVIRONMENT ON ZYGOTIC EMBRYO DEVELOPMENT

1. ABNORMAL COTYLEDONARY DEVELOPMENT
   a. FUSED COTYLDEONS
   b. INCOMPLETE COTYLEDONARY RING FORMATION
2. INHIBITION OF HYPOCOTYL DEVELOPMENT

SUMMARY

1. PRECOTYLEDONARY ZYGOTIC EMBRYOS CAN BE USED TO MODEL THE DEVELOPMENT OF SOMATIC EMBRYOS.
2. SOME ANOMALIES IN SOMATIC EMBRYO DEVELOPMENT APPEAR TO BE CONSEQUENCE OF THE CULTURE ENVIRONMENT.

CONVERSION OF ZYGOTIC EMBRYOS INTO PLANTS

OBJECTIVE:

INVESTIGATE THE NUTRITIONAL, HORMONAL, AND ENVIRONMENTAL CONDITIONS NECESSARY FOR GROWING PRECOTYLEDONARY ZYGOTIC EMBRYOS INTO PLANTS.

UNDERSTANDING CONVERSION OF ZYGOTIC EMBRYOS WOULD:

1. PROVIDE MODEL SYSTEM FOR CONVERSION OF PINE SOMATIC EMBRYOS.

2. ALLOW THE SEPARATION OF CULTURE-INDUCED ARTIFACTS FROM FEATURES OF THE SOMATIC EMBRYOGENESIS PROCESS ITSELF.

RESULTS TO DATE

1. NO PRECOTYLEDONARY ZYGOTIC EMBRYOS HAVE BEEN GROWN INTO PLANTS.

2. CULTURE CONDITIONS CONDUCIVE TO THE GROWTH OF EMBRYOGENIC CALLUS ARE DEARTH TO EMBRYO DEVELOPMENT.

   IN PARTICULAR:
   - HIGH GLUTAMINE LEVELS PROMOTE CALLUS FORMATION.
   - AMMONIUM ION IN EXCESS OF 400 MG/L APPEARS TOXIC.

TREATMENTS STIMULATING EMBRYO
DEVELOPMENT TO THE COTYLEDONARY STAGE

MEDIUM	ADDENDA	DEVELOPMENT, %
WHITE'S	0 +/- GLU	20 ± 13/10 ± 11
	10 MG/L EXTRACT +/- GLU	17 ± 15/15 ± 13
	100 MG/L EXTRACT +/- GLU	13 ± 10/23 ± 15
	1000 MG/L EXTRACT	17 ± 23
	2.6 ABA	3 ± 8

TREATMENTS PRODUCING A CALLUS
RESPONSE IN PRECOTYLEDONARY ZYGOTIC EMBRYOS

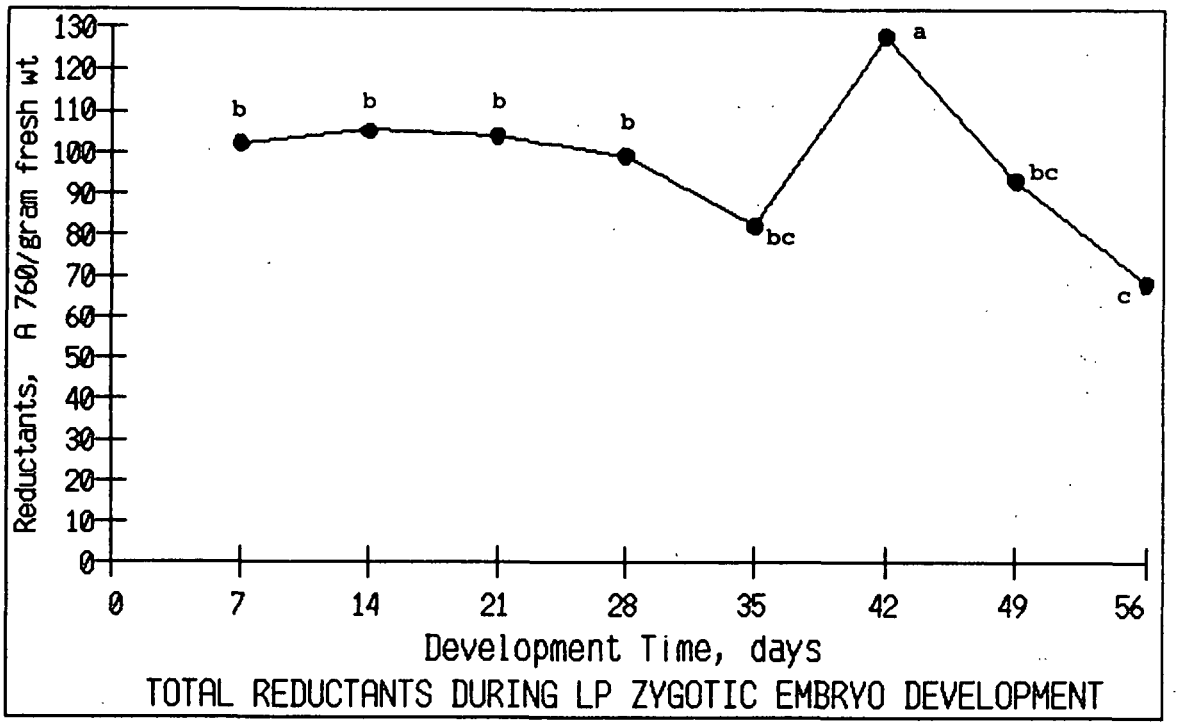
MEDIA	ADDENDA[a]	CALLUS FORMATION FREQUENCY, %
MSG	2.6 ABA	72 ± 10
	2.6 ABA, 6% SUCROSE	68 ± 22
	2.6 ABA, 6% SUCROSE 20% CW	59 ± 30
	EMBRYOS EMBEDDED IN E CALLUS	~100
1/4 MS	400 NH_4	0
	400 NH_4, 2.6 ABA	0
	100 NH_4, 50 GLU	32 ± 17
	100 NH_4	20 ± 24
	0 NH_4, 0 GLU	4 ± 9
	50 GLU	0

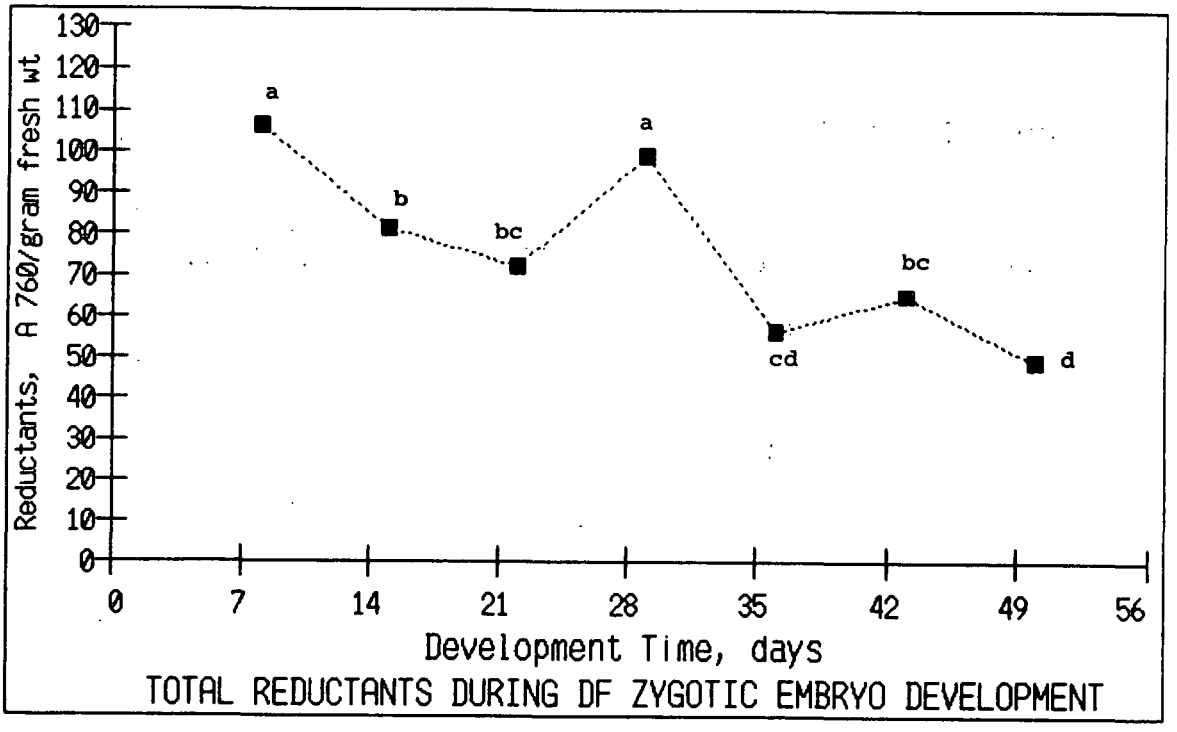
[a]IN MG/L; CW = COCONUT WATER, GLU = GLUTAMINE

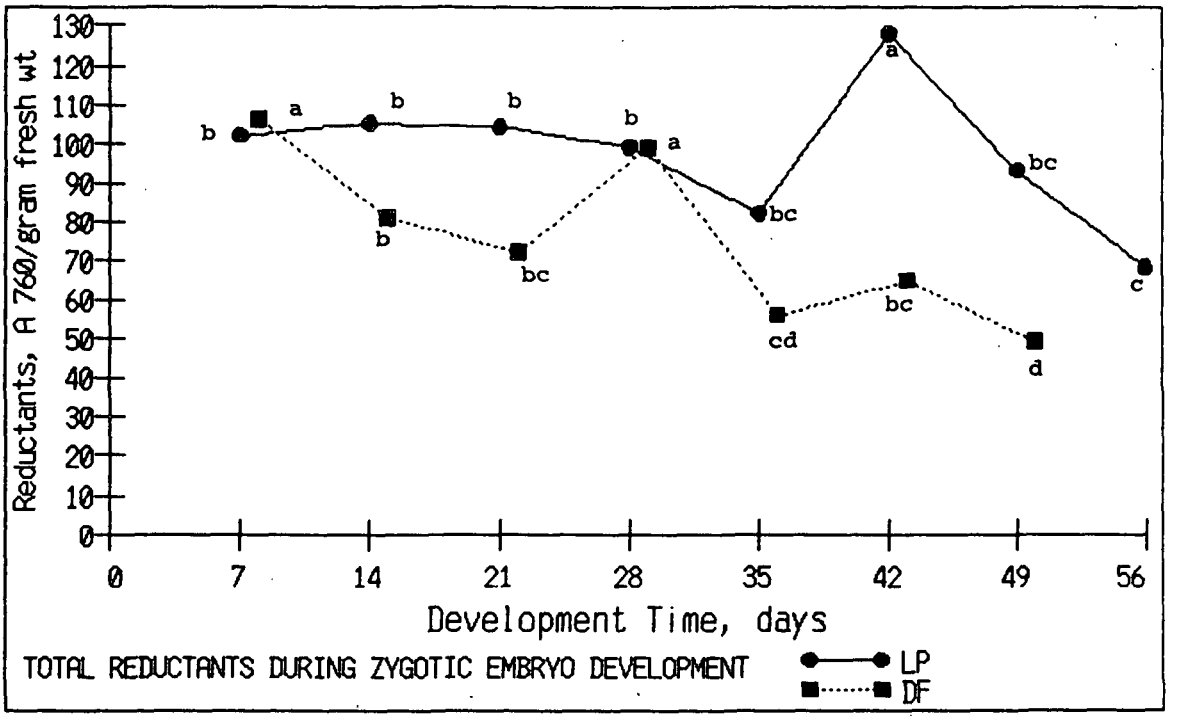
BIOCHEMISTRY OF DEVELOPMENT

BIOCHEMICAL EVENTS

MORRIS JOHNSON


Reductants, A 760/gram fresh wt
130
120
110
100
90
80
70
60
50
40
30
20
10
0
b
b
b
b
bc
a
bc
c
0
7
14
21
28
35
42
49
56
Development Time, days
TOTAL REDUCTANTS DURING LP ZYGOTIC EMBRYO DEVELOPMENT

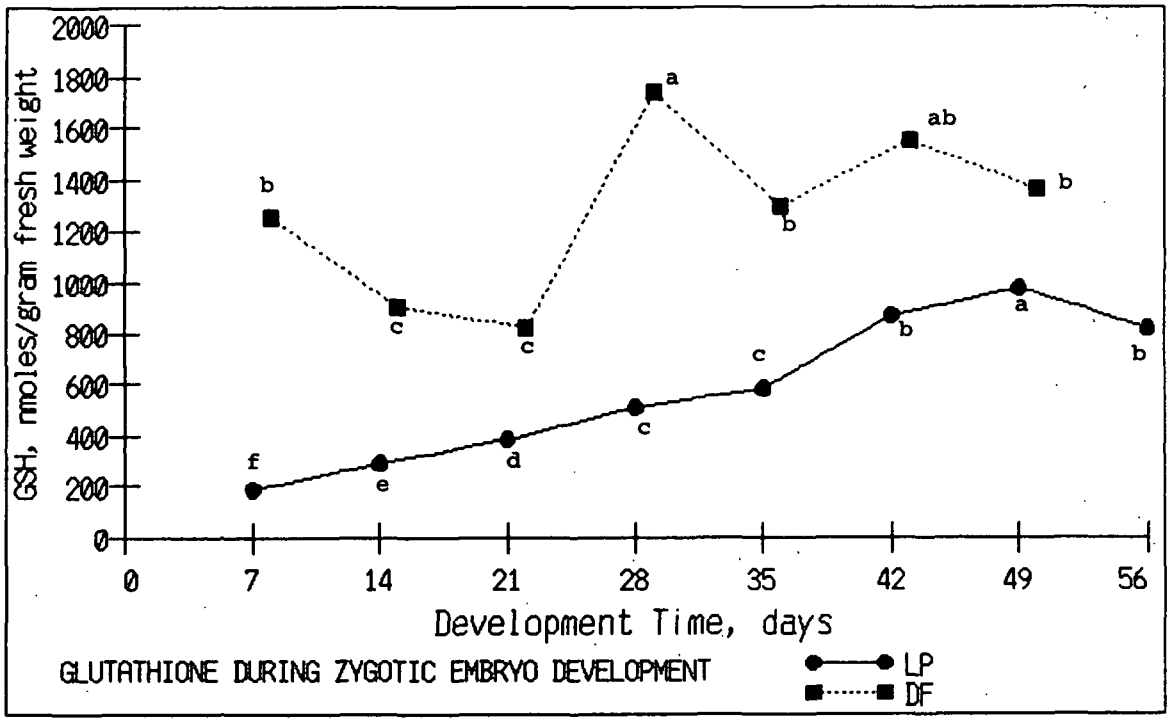

Reductants, A 760/gram fresh wt
130
120
110
100
90
80
70
60
50
40
30
20
10
0
a
b
bc
a
cd
bc
d
0
7
14
21
28
35
42
49
56
Development Time, days
TOTAL REDUCTANTS DURING DF ZYGOTIC EMBRYO DEVELOPMENT



TOTAL REDUCTANTS DISTRIBUTION BETWEEN EMBRYO AND GAMETOPHYTE

Species	Collection Date	Reductants, A_{760}/gram fresh wt*	
		Embryo	Gametophyte
LP	8/10/87	79 ± 6	42 ± 3
DF	7/21/87	174 ± 19	45 ± 3
	7/28/87	135 ± 21	57 ± 7
	8/4/87	114 ± 7	41 ± 2

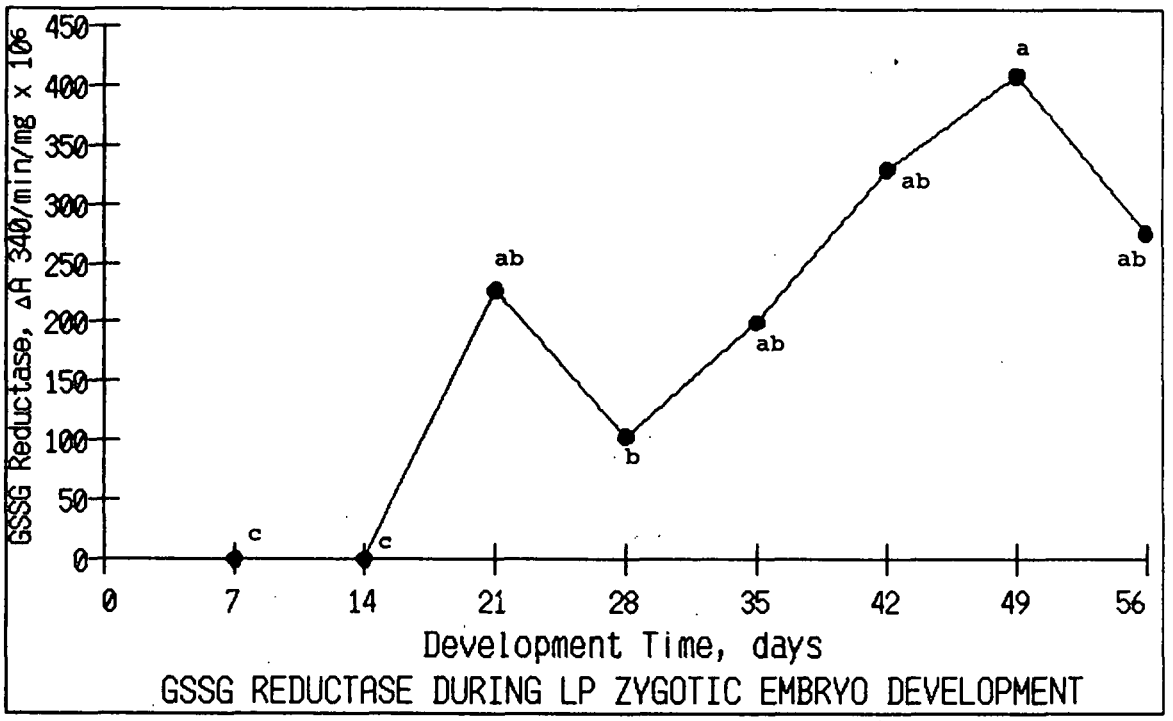
*N = 3; mean ± S.D.

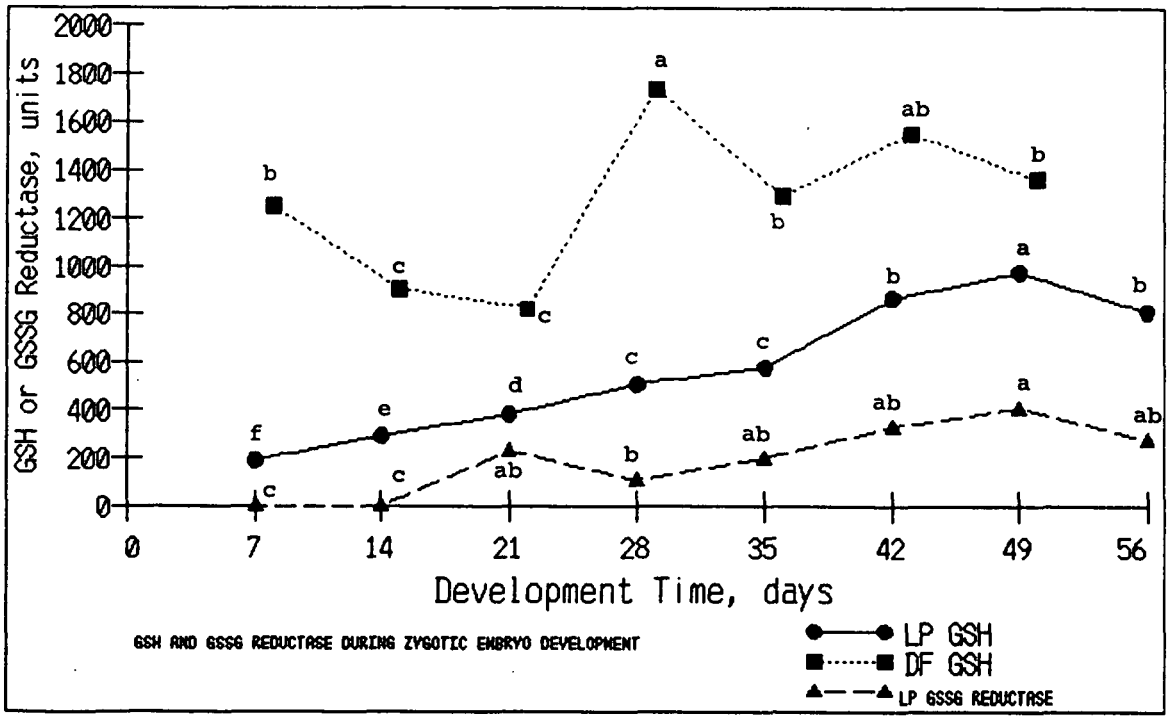


GSH DISTRIBUTION BETWEEN EMBRYO AND GAMETOPHYTE

Species	Collection Date	GSH, nmoles/gram fresh weight*	
		Embryo	Gametophyte
LP	8/10/87	6637 ± 1817	507 ± 101
DF	7/21/87	7354 ± 1287	733 ± 82
	7/28/87	6828 ± 1251	880 ± 31
	8/4/87	5616 ± 560	835 ± 19

*N = 3; mean ± S.D.


GSSG Reductase, ΔA 340/min/mg x 10⁶
450
400
350
300
250
200
150
100
50
0
0
7
14
21
28
35
42
49
56
Development Time, days
GSSG REDUCTASE DURING LP ZYGOTIC EMBRYO DEVELOPMENT


GSH or GSSG Reductase, units
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
7
14
21
28
35
42
49
56
Development Time, days
GSH AND GSSG REDUCTASE DURING ZYGOTIC EMBRYO DEVELOPMENT
LP GSH
DF GSH
LP GSSG REDUCTASE

SOME GSSG REDUCTASE DISTRIBUTIONS

Sample	GSSG Reductase, $\Delta A_{340}/min/mg \times 10^6$*
LP embryo collected 8/10/87	59 ± 58
LP gametophyte collected 8/10/87	314 ± 49
NS seedling needles/cotyledons	29 / 32
NS E callus	29
NS NE callus	complication

*N = 3; mean ± S.D. for LP

SUMMARY - REDUCTANTS

1. ANALYSES OF GSH AND TOTAL REDUCTANTS IN DEVELOPING ZYGOTIC EMBRYOS OF LP AND DF INDICATED LP TO BE THE ODD SPECIES OF THE FOUR CONIFERS THAT HAVE BEEN EXAMINED FOR THESE PARAMETERS.

2. A GSSG REDUCTASE ACTIVITY PEAK COINCIDED WITH THE GSH PEAK DURING LP ZYGOTIC EMBRYO DEVELOPMENT.

SUMMARY - REDUCTANTS (CONTD.)

3. GSH IS MORE CONCENTRATED IN EMBRYOS THAN IN GAMETOPHYTES IN ALL FOUR SPECIES EXAMINED EVEN THOUGH GSSG REDUCTASE WAS MORE ACTIVE IN THE GAMETOPHYTES THAN IN THE EMBRYOS OF LP.

4. THE DIFFICULTY OF CONDUCTING THESE ANALYSES ON ZYGOTIC EMBRYOS PER SE DURING EARLY STAGES OF DEVELOPMENT CURRENTLY LIMITS BUT DOES NOT RULE OUT THE VALUE OF THIS DATA AS BASELINE FOR MONITORING SOMATIC EMBRYO DEVELOPMENT.

SUMMARY - LIPIDS

1. ALTHOUGH NEUTRAL LIPIDS ARE QUANTITATIVELY DOMINANT IN EMBRYO EXTRACTS, AT PRESENT THEY ARE NOT ATTRACTIVE CANDIDATES FOR EXPLOITATION TO FURTHER DEVELOPMENT.

2. THE ABSENCE OF ONE PUTATIVE PHOSPHOLIPID COMPONENT IN NONEMBRYOGENIC WS CALLUS MAY BE AN IMPORTANT LEAD THAT WILL BE HELPFUL IN EMBRYO DEVELOPMENT EFFORTS.

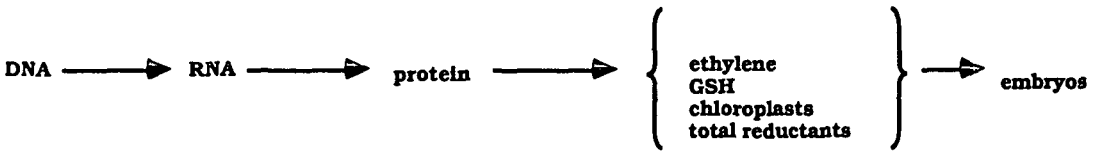
BIOCHEMISTRY OF DEVELOPMENT

PROTEIN COMPOSITION AND COMPARISONS

RUSS FEIRER

"Biochemistry of Development:
Protein composition and comparisons"

Development of new markers and techniques (3/86)



PROTEINS AS MARKERS OF PLANT DEVELOPMENT (11/85)

Hypothesis (11/85)

Synthesis of specific proteins are associated with both natural and in vitro differentiation. These proteins, one identified, may serve as markers of differentiation in our cultures.

Objective (11/85)

- Develop methods to evaluate proteins from small amounts of tissue
- Demonstrate that synthesis of specific proteins are associated with in vitro development
- Attempt to influence specific protein synthesis with growth regulators, polyamines, etc.

Results

- Differences in proteins extracted from embryogenic and non-embryogenic conifer calli are observed
- Changes in proteins extracted from zygotic pine embryos are observed as the embryos mature

Plans

- Compare proteins in somatic embryos to those observed in zygotic embryos
- Use protein data obtained with developing pine zygotic embryos as a baseline to judge the effectiveness of attempts to further the development or "conversion" of our somatic embryos

FIDELITY AND PERFORMANCE

MORRIS JOHNSON

COMPARISON OF GROWTH CHARACTERISTICS OF SOMATIC EMBRYO PLANTS (SE) AND CONTROL SEEDLINGS GROWN FROM ZYGOTIC EMBRYOS (ZE). DATA RECORDED 8/21/87.

PLANT ORIGIN AND CODE	EMBRYOGENIC CALLUS LINE NO.	PLANT HEIGHT, CM	INCREASE IN HEIGHT, %[a]	NO. OF LATERALS WITH ACTIVE GROWTH
SE				
J1	NS1-5	29	87	3
D1	NS1-5	23	229	4
L1	NS1-5	19	280	6
D2	NS1-5	16	220	3
L2	NS1-5	13	117	10
T1	NS1-9	8	23	0
D3	NS1-5	--	dead (7/20/87)	--
D4	NS1-13	--	dead (7/20/87)	--
J3	NS1-5	--	dead (7/30/87)	--
ZE				
Z2	--	37	95	13
Z3	--	36	125	7
Z1	--	27	74	7
Z4	--	26	126	7
Z5	--	22	175	8
Z7	--	17	70	6
Z6	--	--	dead (6/26/87)	--

[a]Increase in height relative to measurement on 5/22/87.

SUMMARY - FIDELITY

1. ALTHOUGH TOTAL HEIGHT GROWTH OF THE GROUP HAS BEEN SOMEWHAT LESS TO DATE, IN OTHER RESPECTS (INCLUDING HEIGHT GROWTH THIS SUMMER) SOMATIC NS PLANTS PLANTED IN 1986 APPEAR SIMILAR TO ZYGOTIC PLANTS OF THE SAME AGE. ONE SOMATIC PLANT WENT DORMANT EARLY THIS SUMMER.

2. BOTH ACID PHOSPHATASE AND PEROXIDASE ISOZYME PATTERNS SHOW THAT ONE OUT OF FIVE SOMATIC PLANTS THAT SHOULD BE IDENTICAL IS NOT. THE SIXTH SOMATIC PLANT OF DIFFERENT ORIGIN, WHICH EXHIBITED THE EARLY DORMANCY, IS ALSO SORTED BY ISOZYME PATTERNS.

EXPLORATORY RESEARCH

SUSPENSION CULTURES

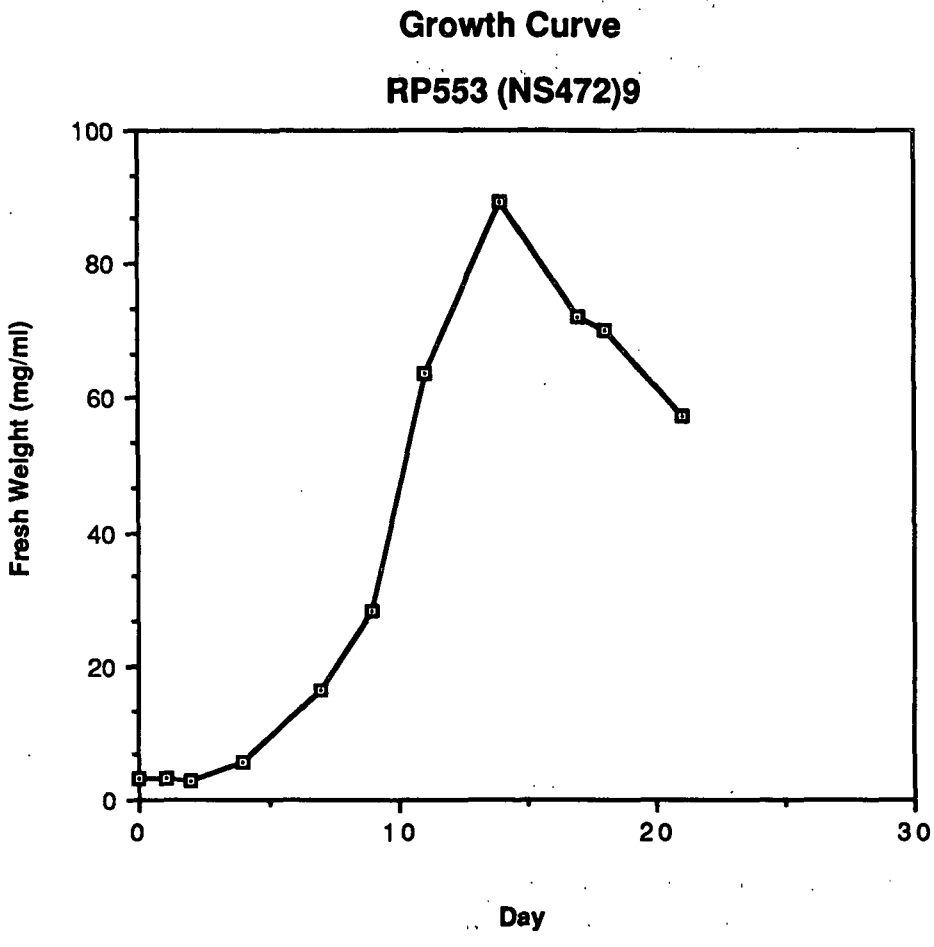
SHIRLEY VERHAGEN

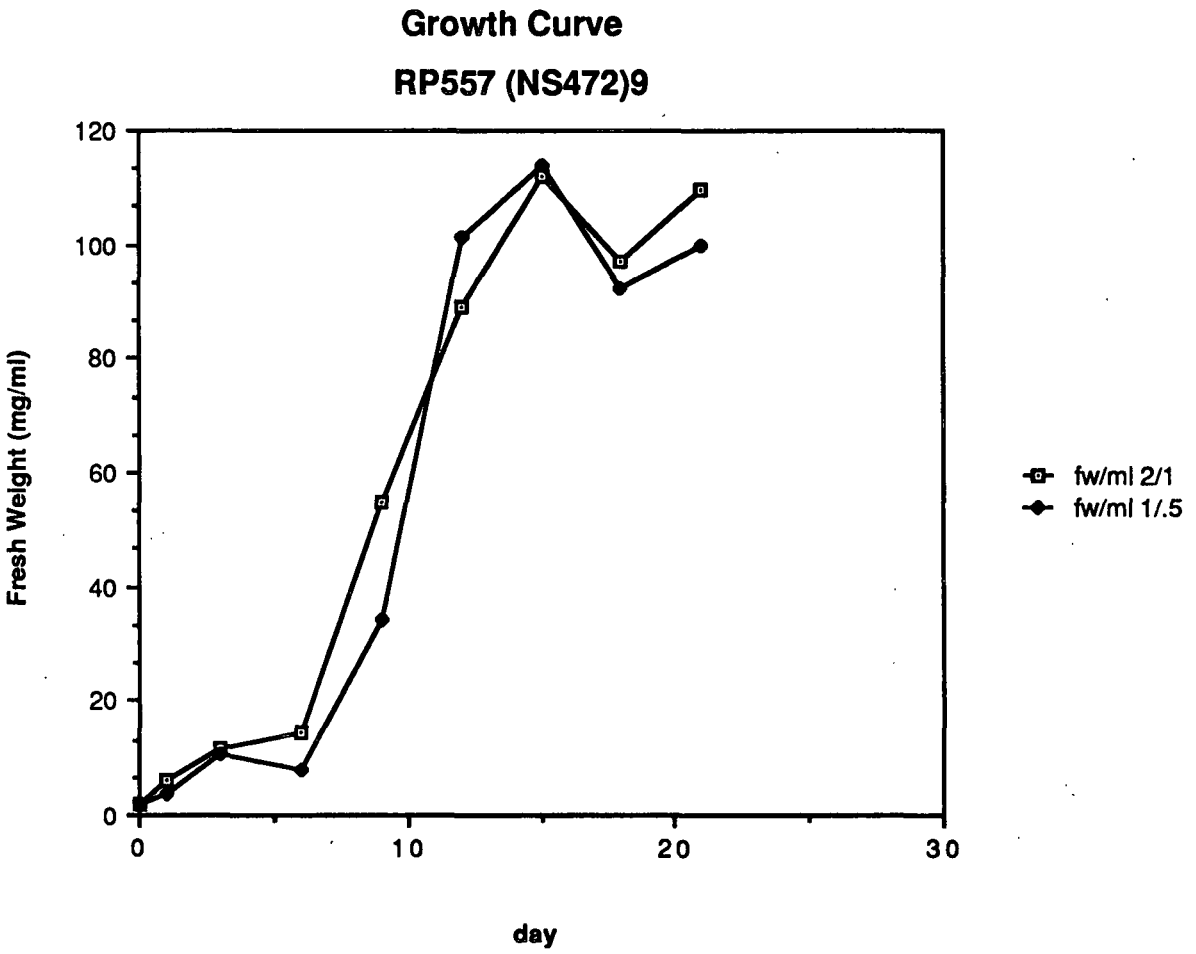
CELL SUSPENSIONS

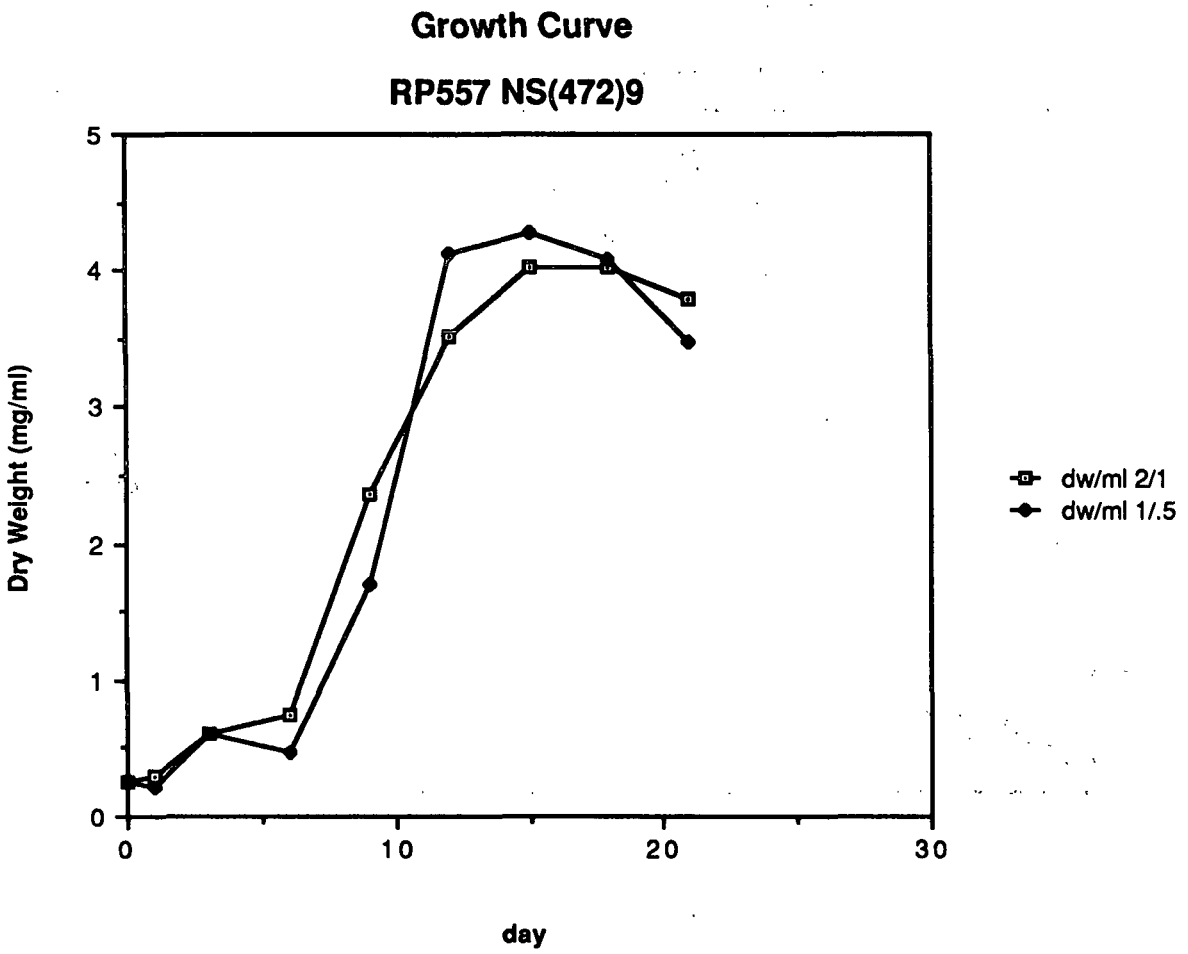
An Alternative Culture System

RESEARCH OBJECTIVES

1. OBTAIN AN EFFICIENT SYSTEM FOR MASS SCALE-UP AND REGENERATION.
2. PROVIDE TISSUE SOURCES READILY UTILIZED FOR PROTOPLAST AND GENETIC IMPROVEMENT STUDIES.
3. INCREASE POTENTIAL FOR FUTURE AUTOMATION AND ARTIFICIAL SEED PRODUCTION.


Growth Curve
RP553 (NS472)9
Fresh Weight (mg/ml)
100
80
60
40
20
0
0
10
20
30
Day

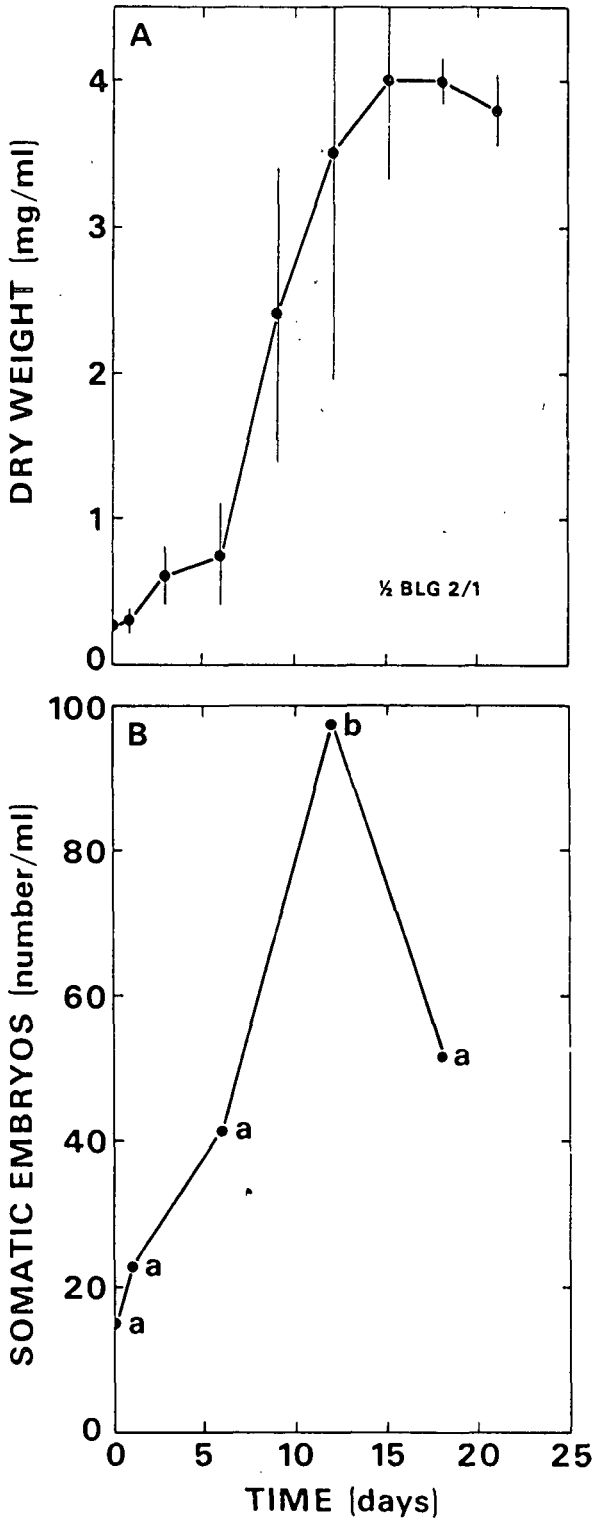

Growth Curve
RP557 (NS472)9
Fresh Weight (mg/ml)
120
100
80
60
40
20
0
0
10
20
30
day
fw/ml 2/1
fw/ml 1/.5

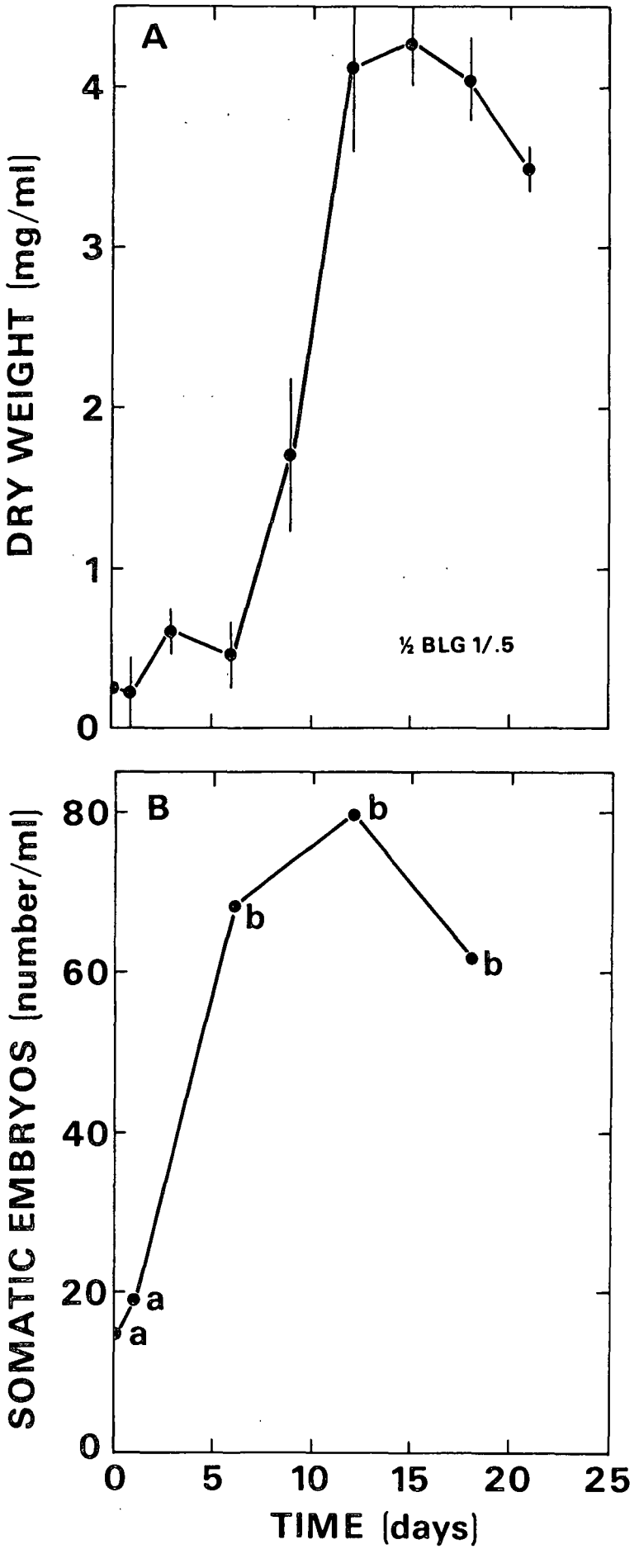

Growth Curve
RP557 NS(472)9
Dry Weight (mg/ml)
5
4
3
2
1
0
0
10
20
30
day
dw/ml 2/1
dw/ml 1/.5

SOMATIC EMBRYOS (NUMBER/ML)

Time, day	Media	
	1/2 BLG 2/1	1/2 BLG 1/.5
0	14.6 ± 11.9	14.6 ± 11.9
1	23.3 ± 4.5	19.0 ± 4.4
6	41.7 ± 17.0	68.3 ± 14.2
12	97.3 ± 13.0	79.7 ± 33.7
18	51.7 ± 36.3	61.7 ± 34.7

$\bar{X} \pm SD$, N = 3


A
DRY WEIGHT (mg/ml)
0
1
2
3
4
½ BLG 2/1
B
SOMATIC EMBRYOS (number/ml)
0
20
40
60
80
100
a
a
a
b
a
0
5
10
15
20
25
TIME (days)


A
DRY WEIGHT (mg/ml)
0
1
2
3
4
½ BLG 1/.5
B
SOMATIC EMBRYOS (number/ml)
0
20
40
60
80
a
a
b
b
b
0
5
10
15
20
25
TIME (days)

SOMATIC EMBRYO DEVELOPMENT
FROM SUSPENSION CULTURES

A. TISSUE
   1. GENOTYPE
   2. SCREENING
   3. INOCULATION DENSITY

B. MEDIA COMPONENTS
   1. BASAL MEDIA (INORGANICS)
   2. HORMONES
   3. ADDITIVES (ORGANICS)

C. CULTURE CONDITIONS
   1. AGAR
   2. LIQUID
      a. FLASKS
      b. ROLLER TUBES
      c. MULTIWELLS (+) CHEESE CLOTH
   3. PHOTOPERIOD

EXPLORATORY RESEARCH

PROTOPLASTS

NAGMANI RANGASWAMY

PROTOPLAST ISOLATION AND CULTURE FROM EMBRYOGENIC-CELL SUSPENSIONS OF NORWAY SPRUCE (MATURE SEEDS)

OBJECTIVES

1. TO FURTHER ESTABLISH THE ORIGIN OF SOMATIC EMBRYO FROM SINGLE PROTOPLAST (CELL).
2. TO USE PROTOPLASTS FOR GENETIC TRANSFORMATION STUDIES.

EXPERIMENTAL PROCEDURE

1. STANDARDIZE TECHNIQUES FOR OBTAINING GOOD YIELD OF VIABLE PROTOPLASTS.
2. MAXIMIZE THE BEST CULTURE CONDITIONS FOR OBTAINING DIVISIONS IN PROTOPLASTS LEADING TO CALLUS FORMATION.

EFFECT OF ENZYME COMBINATIONS AND OSMOTICA ON PROTOPLAST YIELD AND VIABILITY

RUSSELL AND McCOWN'S PROCEDURE FOR POPULUS (1986)	VARDI ET AL. MICROCITRUS (1986)	STRICKLEN ET AL. ON ULMUS HYBRID (1985, 86)	MERKLE AND SOMMER ON YELLOW POPLAR (1986, 87)	NORWAY SPRUCE
LEAF TISSUE	EMBRYOGENIC CALLUS	CALLUS	EMBRYOGENIC CALLUS	CELL SUSPENSIONS FROM EMBRYOGENIC CALLUS
CELLULASE (COOPER BIO-MEDICALS) 0.5% (W/V)	CELLULASE R-10 0.3% (W/V)	CELLULYSIN 1.5% (W/V)	CELLULYSIN 2% (W/V)	VARDI ET AL., 1987
MACERASE (CALBIOCHEM) 0.1% (W/V)	MACEROZYME 0.2% (W/V) DRISELASE 0.1% (W/V)	MACERASE 0.5% (W/V) CALBIOCHEM)	MACERASE 1% (W/V)	MERKLE & SOMMER, 1986, 87
BSA 0.1% (W/V) SUCROSE 550 MM MODIFIED WPM	0.35 M MANNITOL 0.35 M SUCROSE 1/2 MS + TUCKER	POT. DEXTRAN SULFATE 0.5% 0.7 M MANNITOL 1/2 KM MEDIUM	BSA (100 MG/L) MANNITOL 0.5 M $CaCl_2$ $2H_2O$ 500 MG/L MS	

FUTURE RESEARCH PLANS DESIGNED TO INITIATE EMBRYOGENIC CALLUS FROM ISOLATED PROTOPLASTS

1. TEST DIFFERENT CULTURE TECHNIQUES SUCH AS AGAROSE BEADS, ETC., FOR OBTAINING MORE DIVISIONS.
2. TEST DIFFERENT MEDIA COMPOSITIONS AND GROWTH SUPPLEMENTS.

SUMMARY AND DISCUSSION

RON DINUS

PROGRESS: PAC TO PAC

SHORT TERM GOALS	ACCOMPLISHMENTS
DOCUMENT BEST EXPLANT DEVELOPMENTAL STAGES	KNOW BEST STAGES, VARY WITH SPECIES/PROTOCOL
DEVISE/REFINE INITIATION PROTOCOLS	REPEATABLE IN SPRUCES AND PINES MANY SOURCES, GENETIC DIFFERENCES RAISED FREQUENCIES, STILL LOW ROOM FOR IMPROVEMENT D-FIR = STILL TROUBLESOME
IMPROVE PROTOCOLS AND TOOLS FOR DEVELOPMENT/MATURATION	GIVING MAJOR EMPHASIS REFINED PROTOCOLS = SOME PROGRESS E.G., ABA LOOKING GOOD COMPARING ZYGOTIC AND SOMATIC GRANT PROPOSAL
TEST PROCEDURES FOR CONVERSION TO SEEDLINGS	CONTINUING, SLOW BUT SOME SUCCESS
EVALUATE INITIATION PROTOCOLS FOR MATURE EXPLANTS	N & WS = FULLY DEVELOPED SEED TESTING OTHER EXPLANTS
INITIATE STUDIES OF SEEDLING FIDELITY AND PERFORMANCE	DEVELOPING TECHNIQUES: ISOZYMES LOOKING GOOD, OLDER SEEDLINGS GROWING WELL
CONDUCT EXPLORATORY RESEARCH:	
SUSPENSION CULTURES	LOOKING GOOD, EMBRYO NUMBERS INCREASE, BUT DEVELOPMENT SLOW
PROTOPLAST CULTURE	GOOD YIELDS + SOME DIVISION
GENE TRANSFER	SUCCESSFUL AND MOVING, ACTIVE STUDENT GRANT PROPOSAL

SHORT TERM GOALS

REFINE PROTOCOLS FOR INITIATION IN TARGET SPECIES.

ACCUMULATE BASELINE DATA ON HISTOLOGICAL, BIOCHEMICAL, AND MOLECULAR EVENTS IN SOMATIC AND ZYGOTIC EMBRYOGENESIS.

USE BIOCHEMICAL AND MOLECULAR MARKERS/TOOLS TO IDENTIFY EMBRYOGENIC POTENTIAL, IMPROVE INITIATION, AND IMPROVE DEVELOPMENT/MATURATION.

IMPROVE PROTOCOLS FOR DEVELOPMENT/MATURATION AND CONVERSION.

DEVELOP AND TEST TOOLS FOR EVALUATING FIDELITY AND PERFORMANCE.

INCREASE ABILITY TO OBTAIN AND MATURE SOMATIC EMBRYOS IN ALTERNATIVE CULTURE SYSTEMS.

CONTINUE WORK ON INITIATION PROTOCOLS FOR MATURE EXPLANTS.

EXECUTE EXPLORATORY RESEARCH ON PROTOPLAST CULTURE, PROMISING MOLECULAR TECHNIQUES, AND GENE TRANSFER.

SECURE ADDITIONAL EXPLANT MATERIALS FROM SOUTHERN HEMISPHERE.

PUBLISH AND/OR PRESENT PROMPTLY.

GLOSSARY

Adventitious - Roots, shoots, embryos, or other organs or tissues developing in an abnormal position.

Agar - Polysaccharide complex extracted from algae. Used as gelling agent in tissue culture medium.

Agarose - A gelling agent derived from agar: the neutral (charge) fraction of agar.

Archegonium - The flask-shaped container of the ovum (egg cell) of some gymnosperms. The swollen base (venter) contains the egg cell and is surrounded by the neck, with neck canal cells.

Aseptic culture - Surface sterilization of parental explants, free from pathogens, but not necessarily free of internal symbionts.

Asexual reproduction - Reproduction without fertilization. New individuals may develop from vegetative parts such as tubers, bulbs, or rooted stems, or from sexual parts such as unfertilized eggs or other cells in the ovule.

Auxins - A class of plant growth hormones of diverse makeup which cause cell enlargement, apical dominance, and root initiation.

Callus culture - Proliferation from a parental explant of many cells in protoplasmic continuity, but having no equivalence with any normal tissue. Same as tissue culture.

Cell differentiation - Internal chemical or ultrastructural changes preceding or accompanying specialization of function.

Cell suspension - Culture of single cells in moving liquid medium, often used to describe suspension cultures of cells and cell aggregates.

Chloroplast - A membrane-enclosed subcellular organelle containing chlorophyll. Chloroplasts are the sites of photosynthesis. They contain DNA and ribosomes and can replicate.

Clonal propagation - Propagation of a group of plants derived from a single individual (ortet) by asexual reproduction. All members (ramets) of a clone have the same genotype and consequently tend to be uniform.

Conversion - Development of cotyledonary embryo to rooted plantlet.

Coumarins - A class of phenylpropanoid phenolic compounds of which coumarin itself typifies the structures.

Cotyledon - The leaf formed directly from the embryo of an angiosperm or gymnosperm. There may be one (in monocotyledons), two (in dicotyledons), or several (in gymnosperms). They act as storage organs in nonendospermous seeds and as the first photosynthetic organs in endospermous seeds.

Cytokinins - A class of plant growth hormones associated with cell division, assisting with the transmission of the genetic information from the genes to the proteins.

2D TLC - Two-dimensional thin-layer chromatography.

Diploid - Having two sets of chromosomes in the nucleus. One-half of the chromosomes are contributed by one parent, one-half by the other parent. Many higher organisms are diploid except for their sex cells and associated tissue.

EM - Electron microscope.

Embryo - The young plant developing in the megagametophyte from the fertilization of an egg cell, or without fertilization. In aseptic cultures, adventitious embryos show polarization followed by the growth of a shoot from one end and a root from the other end.

Embryogenesis - Initiation of embryoids or embryos from cultured cells.

Embryoid - A cell group approximating an embryo, but having a more random cell arrangement.

Enzyme - A protein molecule that catalyzes a specific chemical reaction.

ER - Endoplasmic reticulum. A system of membranes (originating from the external membrane of the nuclear envelope) that permeates the cytoplasm and that may or may not be covered with ribosomes.

Erosion zone - Zone in the gametophytic tissue below the archegonium that is degraded by the developing embryo.

Eucaryotic cells - Cells with true nuclei bounded by nuclear membranes and which undergo meiosis.

Excise - To cut or isolate callus tissue from its parental explant or to remove adventitious shoots from callus tissue for rooting.

Explant - A plant part excised and prepared for aseptic culture by surface sterilization followed by the exposure of live cells to a nutrient medium.

Fertilization - The normal union of two gametes during sexual reproduction.

Flavonoids - A class of phenolic compounds usually consisting of two hydroxylated aromatic rings joined by a three-carbon chain.

Gametophytic tissue - Haploid tissue of the seed that surrounds the developing embryo during the latter stages of embryogenesis.

Gene - One of the units of inherited material carried on a chromosome; arranged in a linear fashion and indivisible.

Gene pool - Reservoir of genetic variability available for use in genetic improvement of tree species.

Genetic gains - Average improvement in progeny over the mean of the parents.

Genetic variability - The variation existing in a given population (species, for example) with respect to particular genes or arrangement of genes.

Genotype - The genetic makeup of an individual; carried in the chromosomes.

Grana - Association of thylakoids in a stack.

Groundplasm - Homogeneous plasma (matrix) remaining after cell organelles and particles have been excluded.

Haploid - Having the reduced chromosome number, i.e., having one set of chromosomes in the nucleus. This is normal in sex cells, which have only half the number of sets occurring in diploid vegetative cells.

Hormone - Any growth substance which is generally transported to the site of action and can stimulate growth or cell enlargement (auxins), cell division (cytokinins), stem elongation (gibberellins), or can retard growth as in the abscission of leaves (ethylene).

Hybrid vigor - The increase in vigor, size and fertility of a hybrid as compared with its parents, resulting from the union of genetically different gametes and assumed to be due to special recombinations of dominant and recessive genes (heterosis).

Hybridization - The production of offspring of genetically different parents.

Hypocotyl - The part of a seedling axis between the radicle and the cotyledon(s).

Induction - To cause initiation of a plant structure, organ or process.

Inoculation density - "ID" is the volume of cells per unit of medium, i.e.,)L/mL.

Inoculum - A small piece of tissue cut from callus, or a small amount of cell material from a suspension culture placed in contact with fresh medium for continued growth of the culture. Inocula (plural).

Interspecific hybrid - The progeny from matings between species.

Intraspecific hybrid - The progeny from matings within species.

In vitro - Outside the living organism.

In vivo - Within the living organism.

Isozymes - Multiple forms of a single enzyme.

Launch - (Induction), to cause the initiation of a process that will result in the development of a plant structure (shoots, roots, or embryos); sometimes used to describe the log phase of the growth cycle.

Lipids - Any of a group of biochemicals which are variably soluble in organic solvents and barely soluble in water.

Maturation - Development of proembryo to cotyledonary embryo.

Milieu - The whole chemical and physical environment of a culture.

Meristem - A localized group of cells, actively dividing and undifferentiated but ultimately giving rise to permanent tissue such as shoots, roots, wood or bark.

Meristemoid - A localized group of cells in callus tissue, characterized by an accumulation of starch, RNA and protein, and giving rise to adventitious shoots or roots.

Mitochondria - Small bodies in spaces of the cytoplasm. They are spheres or rods, and are the sites of many important aerobic enzymatic processes. The inner layer of the wall is infolded into fingerlike processes.

Morphogenesis - Initiation of organized tissue in callus or suspension cultures.

Nutrient medium - A solid or liquid combination of major and minor salts, an energy source (sucrose), vitamins, hormones, and occasionally other defined or undefined supplements. Usually made up from previously prepared stock solution, then sterilized by autoclaving or filtering through a micropore filter. Media (plural).

Organized tissue - Tissue composed of regularly differentiated cells.

Organelle - A complex cytoplasmic structure of characteristic morphology and function, such as a mitochondrion or plastid.

Organogenesis - Initiation of roots or shoots from callus meristemoids.

Packed cell volume - "pcv" is the volume of cells determined by centrifugation.

Parasexual hybridization - Hybridization resulting from asexual fusion of cells, either diploid or haploid.

Passage - The duration of growth of callus or cell material from one subculture to another.

Photoperiod - Length of daily light cycle.

Plasmalemma - The semipermeable unit membrane surrounding and containing the cell cytoplasm. In plant cells, it is pressed up against the inner surface of the cell wall.

Polyploidy - Having three or more times the haploid number of chromosomes.

Procaryotic cells - Single-celled organisms and reproducing entities that lack a membrane-bound nucleus; they do not undergo meiosis; these include the viruses, bacteria, and blue-green algae.

Proembryo - Embryo in very early precotyledonary stages of development.

Prolamellar body - Semicrystalline structure from which thylakoid membranes arise during chloroplast development in dark grown seedlings.

Proplastids - A group of plastids which are progenitors of chloroplasts.

Protoplast - Spherical cell protoplasm (cytoplasm + nucleus) bounded by a membrane but no cell wall.

Protoplast fusion - Union of two protoplasts into one cell.

Ribosomes - Organelles containing protein and RNA. They are seen as dense particles in electron micrographs. They are found in all types of cells in which protein is being synthesized.

SEM - Scanning electron microscope.

Somatic - Diploid body cells of an organism; those cells other than germ cells.

Somatic cell hybrid - The plant resulting from fusion of protoplasts from somatic cells of genetically different sources.

Subculture - Dividing agar grown callus or liquid cell suspensions for transfer to fresh medium.

Suspension culture - Cells or cell aggregates dispersed and growing in moving liquid medium.

Suspensor - Chain of cells which produces at its extremity the developing embryo.

Tannins - A class of complex phenolic compounds known for their astringency and ability to tan the proteins of animal skins. There are two major types of tannins, the hydrolyzable and the condensed tannins.

TEM - Transmission electron microscope.

Thylakoids - Complex system of flattened membranes within a chloroplast; are often found in stacks to form grana.

Tissue culture - General term for callus and cell cultures.

Totipotency - A cell characteristic in which the cell retains the potential of forming all the cell types of the adult organism.

Ultrastructural - Sublight microscopic, intracellular structure.

Vacuole - A fluid-filled space in a cell. A single vacuole, taking up most of the volume of the cell, present in many plant cells, and containing a cell sap which is isotonic with the protoplasm.

Vegetative cells - Nonreproductive cells such as haploid cells from female gametophytes of conifers or diploid somatic cells.

Vesicle - Small membrane-bound body in the cytoplasm.

Zygote - Fusion product of male and female sex cells or fusion product of protoplasts.

MOLECULAR BIOLOGY GLOSSARY

Agrobacter tumefaciens - Bacterial plant pathogen responsible for crown gall in plants. Harbors a tumor inducing (Ti) plasmid which can be used to transport a foreign gene into a plant cell.

Antibiotic resistance gene - A gene that codes for a protein, which imparts resistance to an antibiotic that allows cells to live in the presence of the drug that would normally kill them.

Bacillus thuringiensis - Bacterium which produces a protein having a strong insecticidal activity. Depending upon the strain of the bacteria, the toxin may exhibit specificity toward Lepidopteran, Dipteran or Coleopteran insect groups.

Bacteriophage - A virus that attacks bacteria; also called a phage.

Base (nucleic acid) - A flat, ring compound that forms part of one of the nucleotide links of a nucleic acid chain. The bases are adenine, thymine, guanine, cytosine and uracil (commonly abreviated A, T, G, C, U).

Base pair - Two bases, one in each strand of a double stranded DNA molecule, which are attracted to each other by weak chemical interactions. Only certain combinations of bases will pair: A-T, G-C and A-U.

Clone - 1. (verb) to undergo the process of creating a group of identical DNA molecules or genes derived from a single source. 2. (noun) a group of genetically identical cells (plants), all derived from a single ancestor.

Cloning vector - Small plasmid, phage or virus DNA molecules used to transfer a DNA fragment or gene from a test tube to a living cell. Some vectors are capable of multiplying inside living cells (bacteria) to result in the multiplication or cloning of the transferred DNA or gene.

Codon - A group of three nucleotides coding for an amino acid.

cDNA (complementary DNA) - DNA synthesized from an RNA template in test tubes using the enzyme reverse transcriptase. The DNA sequence is thus complementary to that of the RNA. cDNA is usually made with radioactive nucleotides and is used as a hybridization probe to detect specific RNA or DNA molecules (genes).

Denature - In reference to DNA, denaturation means conversion of double stranded to single stranded DNA.

Electroinjection - Method of transporting naked DNA into a plant cell having a cell wall using a short duration DC electrical pulse (see electroporation).

Electroporation - Method of transporting naked DNA (gene) into a protoplast using a short duration DC electrical pulse.

E. coli (Escherichia coli) - A bacterium commonly found in the digestive tracts of many mammals, including humans.

Gel electrophoresis - A method for separating molecules based on their size and/or electrical charge. Molecules are forced to run through a gel (e.g., agarose or polyacrylamide) by placing them in an electric field. The speed at which they move depends on their size and/or charge.

Gene cloning - A way to use microorganisms to produce millions of identical copies of a specific region of DNA or gene.

Genetic engineering - The formation of new combinations of heritable material by the insertion of nucleic acid molecules into a vector system so as to allow their stable incorporation into a host organism in which they do not naturally occur.

Genome - May refer to the full genetic complement in the haploid set of chromosomes of a species, but one may speak of nuclear, chloroplastid and mitochondrial genomes

Homologous - Describing regions of DNA molecules that have the same nucleotide sequence. Complementary base pairing can occur between homologous regions in two different DNA molecules.

Intron - A noncoding section of a gene that is spliced out of mRNA before translation into proteins.

Kanamycin - Antibiotic that disrupts protein synthesis in some bacteria and plants.

Lamda - The name of a particular bacteriophage (virus) used extensively in gene cloning.

mRNA (messenger RNA) - RNA that is used by the ribosome to synthesize proteins.

Nick translation - A procedure for radiolabelling DNA in vitro. Used to make a radioactive probe.

Nuclease - A general term for an enzyme that cuts DNA or RNA.

Nucleic acid - DNA or RNA.

Nucleotide - One of the building blocks of nucleic acids. A nucleotide consists of three parts: a base, a sugar and a phosphate.

Plasmid - A small circular DNA molecule found inside bacterial cells. Plasmids reproduce every time the bacterial cell reproduces. Once infected, the bacteria will always contain a plasmid. Some plasmids continue to replicate in a bacterial cell so that a single cell may contain 200 plasmids. Plasmids are thus used to clone a gene.

Probe - A radioactive DNA or RNA molecule used to detect the presence of its complementary strand on an electrophoretic "gel" by hybridization and autoradiography

Promotor - A short nucleotide sequence on DNA recognized by RNA polymerase to initiate transcription (synthesis of mRNA).

Recombinant DNA (rDNA) - Chimeric DNA molecule formed by cutting and splicing of DNA (genes).

Restriction endonucleases - (Restriction enzymes) enzymes that cut DNA at specific nucleotide sequences yielding fragments of various sizes. These enzymes are isolated from a variety of bacteria, and are identified by a three letter abbreviation consisting of the first letter of the genus and the first two letters of the bacterial species name, followed by the strain number (e.g., a particular enzyme isolated from an E. coli strain is designated Eco R1).

RFLPs (restriction fragment length polymorphisms) - DNA molecules from the same gene in two different individuals may differ slightly, and fragments of different length are formed when the gene is digested with a restriction enzyme. Since unequal-sized fragments travel at different speeds in an electrophoresis gel, the two fragments visualized by a radioactively-labelled homologous probe would appear as different bands on the gel. This is a RFLP.

Reverse transcriptase - An enzyme purified from tumor viruses that synthesizes DNA complementary to an RNA template.

RNA - Ribonucleic acid. RNA is usually single stranded.

RNA polymerase - The enzyme responsible for making RNA complementary to a DNA template. RNA polymerase binds at specific nucleotide sequences (promotors) in front of genes in DNA. It then moves through a gene and makes an RNA molecule that contains the information contained in the gene.

Sequence - The order of the nucleotides in the DNA or RNA chain.

Splicing - Removal of introns from the "immature" form of eukaryotic mRNA. Carried out in the nucleus of the cell.

Template - A pattern of nucleotide sequences in DNA or RNA used by polymerases to specify the sequence in a new polymer by complementarity.

Tetracycline - An antibiotic that kills bacteria by blocking protein synthesis.

Ti plasmid - The plasmid carried by the bacterium Agrobacter tumefaciens which is used to carry foreign genes into a plant cell.

Transcription - The process of converting information in DNA into information in RNA. The copying of a gene into RNA. RNA polymerase is the enzyme that executes this conversion of information.

Transformation - The process whereby a cell takes up free DNA such that the free DNA (gene) becomes a permanent part of the cell's genome.

Translation - The process of converting the information in mRNA into protein. Also called protein synthesis.

Transposon - A short section of DNA capable of "jumping" to another region of a chromosome or to a different chromosome.

Transposon tagging - Method of using a transposon to locate a gene. When a transposon inserts into a chromosome, it causes a knockout mutation leading to a distinct mutant phenotype. A radioactive probe made from this transposon can then be used to identify the DNA sequence (gene) into which it had been inserted. The gene can then be localized on a gel and perhaps on a particular chromosome from the mutant plant. In short, the mutated gene is tagged or made identifiable by the transposon.

AMINO ACIDS ABBREVIATIONS

ala	alanine
arg	arginine
asn	asparagine
asp	aspartic acid
cit	citrulline
cys	cysteine
q-aba	aminobutyric acid
gln	glutamine
glu	glutamic acid
gly	glycine
his	histidine
hyp	hydroxyproline
ile	isoleucine
leu	leucine
lys	lysine
met	methionine
orn	ornithine
phe	phenylalanine
pro	proline
ser	serine
thr	threonine
trp	tryptophan
tyr	tyrosine
val	valine

CUMULATIVE LIST OF ABBREVIATIONS

AA	Ascorbic acid
2,4-D	2,4-Dichlorophenoxyacetic acid
ABA	Abscisic acid
ACC	1-Aminocyclopropane-1-carboxylic acid
ADC	Arginine decarboxylase
ADP	5'-Adenosine diphosphate
AMP	5'-Adenosine monophosphate
ANOVA	Analysis of variance
AOA	Aminooxyacetic acid
AOAA	Aminooxyacetic acid
AOPP	a-Aminooxy-b-phenylpropionic acid
ATP	Adenosine triphosphate
AVG	Aminoethoxyvinylglycine
BA	Benzylaminopurine = benzyl adenine
BAP	Benzylaminopurine = benzyl adenine
BLG	Brown and Lawrence medium + gln
BSO	Buthionine sulfoximine
cAMP	3',5'-Cyclic adenosine monophosphate
CBM	Bornman medium
C/N	Carbon/nitrogen
D	Dark
DCR	Durzan sugar pine medium
DF	Douglas-fir
DFMA	a-difluoromethylarginine
DFMO	a-difluoromethylornithine
DCHA	Dicyclohexylammonium sulfate
DHA	Dehydroascorbic acid
dSAM	Decarboxylated SAM
DW	Dry weight
E	Embryogenic
E_c	Embryogenic callus
EDTA	Ethylenediaminotetraacetic acid
E_i	Embryonal initial
FAA	Free amino acid(s)
FTIR	Fourier transform infrared
FW or fr.wt.	Fresh weight
G-1-P	Glucose-1-phosphate
GA	Gibberellic acid (gibberellin)
GC	Gas chromatography
GC/MS	Gas chromatography/mass spectrometry
GD	Gresshof and Doy medium
GSH	Glutathione (reduced)
GSSG	Glutathione (oxidized)
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HFBI	Heptafluorobutyrylimidazole
HFSE	High frequency somatic embryogenesis
HM	Hakman medium
HPLC	High performance liquid chromatography
IAA	Indoleacetic acid

IBA	Indolebutyric acid
IPA	Isopentenylaminopurine = 2iP
L	Larch, light or liter
LFSE	Low frequency somatic embryogenesis
LM	Litvay medium
LP	Loblolly pine
lx	Lux
MEOI	Methyleneoxindole
MES	Morpholinoethane sulfonic acid
MOI	Methyloxindole
MOPS	Morpholinopropane sulfonic acid
MGBG	Methylglyoxal bis-guanyl hydrazone
MS	Murashige and Skoog medium
NAA	Naphthalene acetic acid
NAD^+	Nicotinamide adenine dinucleotide (oxidized)
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidized)
NE	Nonembryogenic
NBT	Nitrobluetetrazolium
NOAA	Naphthoxyacetic acid
NS	Norway spruce
OBHA	o-benzylhydroxylamine
ODC	Ornithine decarboxylase
P	Putrescine or phosphate
PAL	Phenylalanine ammonia lyase
pcv	Packed cell volume
PEG	Polyethylene glycol
PO	Pond pine
PP	Pitch pine
PPi	Pyrophosphate
ProA	Proanthocyanidin
RP	Red pine or research plan
S	Suspensor
SAM	S-adenosylmethionine
Sd	Spermidine
S_e	Somatic embryo
S_i	Suspensor initial
SIM	Selective ion monitoring
Sp	Spermine
TLC	Thin-layer chromatography
TrpAM	Tryptamine
2iP	Isopentenylaminopurine
UDP	Uridine diphosphate
UDPG	Uridine diphosphate glucose
UTP	Uridine triphosphate
WC	Wild carrot
WCM	Wild carrot medium
WP	White pine
WS	White spruce

STATUS OF PUBLICATIONS AS OF 10/87

PUBLISHED OR IN PRESS:

1. Becwar, M. R.; Noland, T. L.; Wann, S. R. A method for quantification of the level of somatic embryogenesis among Norway spruce callus lines. Plant Cell Rpts. 6:35-38(1987).

2. Becwar, M. R.; Noland, T. L.; Wann, S. R. Somatic embryo development and plant regeneration from embryogenic Norway spruce callus. Tappi J. 70(4): 155-160(1987).

3. Becwar, M. R.; Verhagen, S. A.; Wann, S. R. The frequency of plant regeneration from Norway spruce somatic embryos. Proceedings 19th Southern Forest Tree Improvement Conf., College Station, TX, June 16-18, 1987. The National Technical Information Service, Springfield, VA.

4. Boston, R. S.; Becwar, M. R.; Ryan, R. D.; Goldsbrough, P. B.; Larkins, B. A.; Hodges, T. K. Expression from heterologous promoters in electroporated carrot protoplasts. Plant Physiol. 83:742-746(1987).

5. Feirer, R. P. (1987) Plant growth: The role of polyamines. In press - invited contribution to McGraw-Hill Encyclopedia of Science and Technology.

6. Johnson, M. A.; Carlson, J.; Conkey, J.; Noland, T. Biochemical changes associated with zygotic pine embryo development. Journal of Experimental Botany 38:518-24(1987).

7. Johnson, M. A.; Wann, S. R.; Noland, T. L.; Carlson, J. A. Biochemical differences between embryogenic and nonembryogenic callus of Picea abies (L.) Karst. Plant Cell Reports 6:39-42(1987).

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12. Wann, S. R. Somatic embryogenesis in woody species. Hort. Rev. (in press).

SUBMITTED:

1. Becwar, M. R.; Wann, S. R.; Johnson, M. A.; Verhagen, S. A.; Feirer, R. P.; Nagmani, R. Development and characterization of in vitro embryogenic systems in conifers. In Somatic Cell Genetics of Woody Plants. M. R. Ahuja, ed. Int. Union of Forest Res. Org. Workshop. Martinus Nijhoff Publ., Dordrecht, The Netherlands. (Submitted)

2. Johnson, M. A.; Carlson, J. A.; Conkey, J. H. Endogenous antioxidants and energy considerations in pine vs. wild carrot cell suspension cultures. Plant Science. (Submitted 1987)

3. Slocum, R.; Bitonti, A.; McCann, P.; Feirer, R. (1987) DFMA metabolism in tobacco and mammalian cells: Inhibition of ornithine decarboxylase activity following arginase-mediated hydrolysis of DFMA to DFMO. Biochem. J. (Submitted)

4. Wann, S. R.; Wyckoff, G. W.; Wyckoff, J. L. A tissue culture solution to a forestry problem - The propagation of a tetraploid European aspen. (Submitted to Tree Planters' Notes.)

5. Wann, S. R.; Becwar, M. R.; Kroll, L. F.; Nagmani, R.; Kriebel, H. B. Factors influencing the initiation of embryogenic callus in Pinus strobus L. (Submitted to Plant Cell Tissue and Organ. Culture.)

OTHERS FOR INFORMATION:

1. Conners, T. E. and McLain, T. E. Modeling moisture gradient effects on bending properties. In press (1987). To be published in Wood and Fiber Science.

2. MacGregor, M. A. and Conners, T. E. Image analysis of an LWC paper reveals wire mark in the print density variations. Tappi J., Vol. 70, no. 9:95-100 (Sept. 1987).

3. Wyckoff, Gary W.; Einspahr, Dean W.; Dinus, Ronald J. Growth and survival of hybrid poplar and Populus deltoides clones in East Central Wisconsin. Proceedings Fifth North Central Tree Improvement Conference, Fargo, ND, August 10-12, 1987. (In press)

CONFERENCE PRESENTATIONS AND PARTICIPATION:

Southern Forest Tree Improvement Conference
College Station, TX, June 16-18, 1987

Becwar, M. R.; Verhagen, S. A.; Wann, S. R.
The frequency of plant regeneration from Norway spruce somatic embryos.

Genetic Manipulation of Woody Plants Conference
Michigan State University, June 21-25, 1987

Becwar, M. R.; Wann, S. R.; Kriebel, H. B.
Initiation of embryogenic callus in Pinus strobus (eastern white pine) from immature embryo explants.

Becwar, M. R.; Wann, S. R.; Johnson, M. A.
Complete plant regeneration from Norway spruce somatic embryos: Maturation, germination, and renewed vegetative growth from resting buds.

Feirer, R. P.; Wann, S. R.; Becwar, M. R.; Nagmani, R.; Carlson, J.
A comparison of embryogenic and nonembryogenic conifer calli: Chloroplast ultrastructure and protein synthesis.

Wann, S. R.; Johnson, M. A.; Feirer, R. P.; Becwar, M. R.; Nagmani, R.
Biochemical differences between embryogenic and nonembryogenic callus of conifers.

Becwar, M. R. Invited moderator of session.

Annual Meeting of the American Society of Plant Physiologists
St. Louis, MO, July, 1987

Feirer, R. P.; Wann, S.; Becwar, M.; Foxgrover, E.
The occurrence of proplastids in somatic embryos of Norway spruce and carrot.

Johnson, M. A.
The effect of buthionine sulfoximine on maturation of Norway spruce somatic embryos.

Slocum, R. D. and Feirer, R. P.
3H-DFMA metabolism in tobacco: Nonspecific, arginase mediated inhibition of ornithine decarboxylase activity.

CONFERENCE PRESENTATIONS AND PARTICIPATION (CONTD.):

International Union of Forest Research Organizations -
Molecular Genetics Working Group Conference
Chalk River, Ontario, Canada, June 16-18, 1987

Feirer, R. P.
Chloroplast ultrastructure and gene expression in embryogenic conifer callus.

Forest Products Research Society 41st Annual Meeting
Louisville, KY, June 21-24, 1987

Feirer, R. P.
Recombinant DNA research in the field of forestry.
(Invited plenary speaker)